W-Pos138

A NUCLEAR MAGNETIC RESONANCE STUDY OF THE INTERACTION OF Mn^{++} IONS WITH DNA.

John M. Stevens, Himanshu Oberoi and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06457

Proton and phosphorus nuclear magnetic resonance (NMR) spectroscopy has been used to investigate the interaction of Mn^{++} with the dodecamers 5'-(CGCGAATTCGCG)-3' (I) and 5'-(CGCGAGCTCGCG)-3' (II). Longitudinal relaxation rates and linewidths of NMR resonances of individual phosphate groups, hydrogen-bonded imino protons and non-exchangable protons have been monitored as a function of Mn^{++} concenetration in 10 mM Bis-Tris- Propane buffer with 50 mM NaCl, pH 6.8 at 25°C. Several proton resonances in the two dodecamers have been found to be preferentially broadened by Mn^{++}. They originate from: G4-H8 and A5-H2 in (I), and G4-H8, A5-H8, G6-H8 and A5-H2 in (II). No preferential effects have been observed for the Mn^{++}-induced broadening of the ^{31}P NMR resonances of the phosphate groups. These results indicate that the binding of Mn^{++} to the bases in the two dodecamers is influenced by base sequence. Unique sites of interaction exist at 5'-GA-3' and 5'-AG-3' steps. This selectivity could involve a different mode of binding and/or affinity for the metal ion at these sites.

(Supported by a research grant from the NSF)

W-Pos139

PROTON EXCHANGE AND BASE-PAIR OPENING KINETICS IN d(CGCGAATTCGCG) AND RELATED DODECAMERS.

James G. Moe and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut 06457

We have determined the lifetimes of individual base-pairs in the DNA oligonucleotide 5'-d(CGCGAATTCGCG)-3' and in three other dodecamers with symmetrical base substitutions in the fourth and ninth positions. The lifetimes were obtained from the dependence of selective longitudinal relaxation times and linewidths of the imino proton resonances on the concentration of base catalyst (Tris) at 25°C. The results indicate that proton exchange in these DNA dodecamers is dominated by the individual opening of single base-pairs. The lifetimes of the base-pairs in the EcoRI recognition site 5'-d(GAATTC)-3' are less than 100 ms. The lifetimes of the central A·T base-pairs are dependent on base sequence and correlate well with the bending properties of the sequences. They are greatly increased in the dodecamer 5'-d(CGCAAATTTGCG)-3' which contains a tract of six contiguous A·T base-pairs including a 5'-AT-3' step. The results are discussed in terms of the conformational features of the poly(A) family of B-DNA.

(Supported by a research grant from the NSF)

W-Pos140

A NEW TYPE OF GA MISMATCH BASE PAIR IN A PURINE-RICH DNA DUPLEX.

Ying Li and W. David Wilson, Department of Chemistry, Georgia State University, Atlanta, GA and Gerald Zon, Applied Biosystems, Foster City, CA

1D and 2D NMR experiments indicate that the oligomer d($A_1T_2\underline{G_3}\underline{A_4}G_5C_6\underline{G_7}\underline{A_8}A_9T_{10}A_{11}$) forms a ten base-pair purine-rich duplex with four GA base pairs (underlined) and a 3' unpaired base (A_{11}). CD and 2D NMR results are consistent with a global right-handed B-helical structure. Imino proton NMR chemical shifts and exchange results indicate that the G imino protons of the GA mismatches are not hydrogen bonded but are stacked into the helix. Substitution of G by I in the GA base pairs causes a dramatic decrease in duplex stability and indicates that hydrogen bonding of the G amino group is critical for duplex stability. Cross-peaks in 2D NMR NOESY spectra indicate that A_4 must extensively stack over A_8 from the opposite chain and G_3 over G_7 at the GA base pairs. A molecular model of the unusual duplex was constructed with previously unobserved GA base pairs containing A-NH_2 to GN3 and G-NH_2 to AN7 hydrogen bonds and B-form base pairs at the 5' and the 3' sides of the GA pairs (sequence for modeling: d(ATGAGC)).

W-Pos141

SOLUTION STRUCTURE OF AN UNUSUALLY STABLE RNA HAIRPIN: GGAC(UUCG)GUCC

C. Cheong, G. Varani, I. Tinoco, Jr., Chemistry Department and Laboratory of Chemical Biodynamics, U. of California, Berkeley

The solution structure of a very stable RNA hairpin has been investigated by homo- and heteronuclear 2D-NMR spectroscopy. The dodecamer folds into a hairpin with a tetra-nucleotide loop and displays unusual structural and thermodynamic properties. The loop C(UUCG)G occurs exceptionally often in a variety of RNAs and may serve as a nucleation site for RNA folding or as a protein recognition site. A high resolution structure has been derived from interproton distances and scalar coupling constants. Several novel features are found: 1. The loop nucleotide, G(8), adopts a *syn*-conformation. 2. The sugar conformations of the two loop nucleotides, U(6) and C(7), are C2'-*endo*. 3. Several favorable base-phosphate contacts are observed in the loop, but no base-base hydrogen bonding interactions are found. These results are supported by studies on a mutant hairpin, 5'-GGAC(UUUG)GUCC, of diminished thermodynamic stability (The melting temperature is 6.7°C lower). The very compact and stable structure of the loop may explain why reverse transcriptase cannot read through the loop.

W-Pos142

SEQUENCE DEPENDENCE OF THE LENGTH OF B TO Z JUNCTIONS. Z. DAI, E. EVERTSZ, G. A. THOMAS AND W. L. PETICOLAS, Department of Chemistry, University of Oregon, Eugene, Oregon 97403

A series of oligonucleotides of the form $d(CG)_N(A)_M$ and their conjugates, $d(T)_Md(CG)_N$ have been synthesized and their conformation determined in 0.5M and saturated NaCl solution using Raman spectroscopy. Each of the individual components of a duplex self-associates because of the repeating CG portions of the chain. This forms a structure in which the CG sections are in a double helix with the single strands of either nonbonded A or nonbonded T loose at each end. These CG sections of the individual oligonucleotides go into the Z form in 6 M salt solutions. All of the individual oligonucleotides form double helical duplex structures with their conjugates in aqueous solution. These are always in the B form at low salt concentrations. For values of M = 5 and N = 3 the entire duplex remains in the B form in saturated salt solutions. However for values of $N \geq 4$, the CG portion of the helix goes into the Z form under high salt conditions. The d(A)5.d(T)5 portion of the helix remains in the B form giving rise to a B to Z conformational junction. The junction occurs entirely in the CG portion of the helix and the junction length appears to decrease with increasing N. The minimum junction length has not been determined but Raman band intensities indicate that it must be 3 base pairs or less. The determination of the length of the junction is obtained from the relative intensity of the B and Z form marker bands at 688 and 625 wavenumbers. If $NiCl_2$ is added to a solution of a junction in saturated salt solution, the entire duplex including the d(A)5.d(T)5 sequence goes into the Z form.

W-Pos143

FERGUSON PLOTS OF NORMAL AND ANOMALOUS DNA FRAGMENTS IN POLYACRYLAMIDE GELS Holmes, D. L. and Stellwagen, N.C., Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

Ferguson plots (semilogarithmic plots of mobility as a function of gel concentration) have been determined for multimers of two 147 bp DNA restriction fragments in polyacrylamide gels. Linear Ferguson plots are obtained for both the normal and anomalously migrating multimer ladders in polyacrylamide gels ranging from 4.6 to 10.5% in concentration. However, the slopes of the lines are steeper for the anomalous multimers, indicating that the effective pore size of the gel is smaller for these fragments. Semilogarithmic plots of the mobility as a function of cross-linker concentration or the concentration of added linear polyacrylamide are not linear, indicating that the effective pore size of the gel does not change in a linear manner with either of these variables. Gel pore sizes have been estimated for selected concentrations of each of these variables from the extended Ogston theory of pore size distribution.

W-Pos144

PHYSICAL STUDY OF rRNA RECOGNIZED BY PROTEIN L11 Lance Laing, David Draper, Dept. of Chem., The Johns Hopkins Univ., Balt., MD 21218

Bases 1052-1108 of *E. coli.* 23S rRNA, which make up a highly conserved region called the "GTPase center," are specifically recognized by protein L11 and the thiostrepton (TS) family of antibiotics. We are attempting to define the rRNA tertiary structure recognized by L11. The DNA coding for the rRNA site has been cloned into *E. coli.* plasmids and milligram quantities of the RNA have been produced for UV monitored thermal denaturation studies. Wild type sequences as well as sequence variant rRNA fragments defective in L11 binding have been melted under various cationic conditions. Three melting transitions have been detected. Most of the secondary structure melts in one concerted transition which is very sensitive to Mg^{2+}, and is probably indicative of a tertiary structure in the molecule. At low Mg^{2+}, a high temperature transition remains which is identified with a very stable hairpin. A third transition, affected by TS binding, cannot be identified with any secondary structure.

W-Pos145

PROTEIN L11 RECOGNITION OF A CONSERVED 23S rRNA DOMAIN. Patricia C. Ryan and David E. Draper, Dept. of Chemistry, The Johns Hopkins Univ., Baltimore, Md. 21218.

The ribosomal protein L11 from *E. coli* specifically binds to the highly conserved "GTPase center" of the 23S rRNA. A filter binding assay has been used to measure the thermodynamics of L11 binding to several different rRNA fragments. A 57 nt fragment (C1052-U1108) contains all the protein recognition features. Binding is weakly dependent on temperature and K^+ but strongly requires multivalent cations, suggesting a requirement for a specific tertiary structure in L11 recognition. *E. coli* L11 recognizes the homologous RNA structure from a variety of large subunit rRNAs. A number of mutations at bases conserved within these RNAs have been made and tested for L11 binding; only a few have a strong effect on L11 binding affinity. An antibiotic, thiostrepton, also binds to the same rRNA fragment; at least one mutation which strongly affects thiostrepton binding has almost no effect on L11 binding. These mutagenesis experiments should map not only base-specific recognition features but also tertiary structures required for recognition site folding.

W-Pos146

DNA ASSOCIATIONS: PACKING CALCULATIONS IN A-, B-, AND Z-DNA STRUCTURES.

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A detailed theoretical study has been made to understand the modes of DNA - DNA interactions by applying principles of hard sphere contact criteria. Two DNA molecules of similar standard structure (A-, B-, or Z-DNA) and length (10-30 residues) are oriented side-by-side and their relative positions are controlled by translations along and rotations about specific axes. Short atomic contacts between the pair of interacting DNA structures are assessed and the contact-free conformations are studied in detail. Computed hard sphere packing patterns are found to be similar to the packing arrangements observed in DNA crystals. Our theoretical studies also show that the length and morphology of the interacting DNA chains play a major role in packing. The formation of "super" major and minor grooves is noticed in the assembly, especially in longer pieces. (Supported by USPHS grant GM20861).

W-Pos147

NMR STUDIES OF OLIGONUCLEOTIDE DUPLEXES CONTAINING A BULGED BASE.

Karol Maskos and Kathleen M. Morden, Dept. of Biochemistry, Louisiana State University, Baton Rouge, LA 70803

The structure of DNA duplexes containing helical interruptions resulting from the presence of an unpaired base on one strand is important in understanding the mechanism of frameshift mutagenesis. The structural features of seven oligonucleotide duplexes with the sequence d(GCGA$_2$XA$_2$GCG)•d(CGCT$_2$YT$_2$CGC). Duplex 1-3: X= C,T, or G and Y not present; duplex 4-6: Y=C, A or G and X not present; and the parent duplex 7: X and Y both not present, have been investigated using ^{1}H NMR techniques. Analysis of the NOESY and COSY spectra shows that all of these duplexes are in the B-family and the unpaired base is unstacked or stacked in the helix depending on the sequence. The influence of the unpaired base on the duplex formation, stability, and local geometry will be discussed and compared with the parent duplex. Supported by N.I.H. Grant GM38137 and Louisiana Education Quality Support Fund LEQSF(86-89)-RD-A-12.

W-Pos148

STRUCTURAL STUDIES OF B-Z JUNCTIONS.

Richard D. Sheardy, Dept. of Chemistry, Seton Hall University, & Stephen A. Winkle, Dept. of Chemistry, Florida International University.

Recently we reported that the B-Z junction present in a 16 base pair DNA oligomer encompassed three base pairs and showed flexibility properties different from the flanking B and Z regions [R.D. Sheardy, & S.A. Winkle, BIOCHEMISTRY 28, 720-725(1989)]. We have ligated oligomers containing potential B-Z junctions to create DNA molecules possessing multiple junctions. The mobilities of these molecules through polyacrylamide gels at various acrylamide and cobalt hexamine concentrations indicate apparent sizes that are less than actual sizes. The junction regions in these molecules containing multiple junctions, as well as the original molecule possessing only one junction, have been probed using Bal 31, exonuclease III, lambda exonuclease, the restriction nuclease Mbo I, and dam methylase. The mobility shifts and the results of the enzyme assays all suggest that the junction regions have a perturbed or unusual structure. (This work supported by NSF Grant DMB-89-96232 (RDS) and a FIU Provost Summer Fellowship (SAW)).

W-Pos149

INFLUENCE OF SINGLE STRAND LOOP SEQUENCE ON THE B TO Z TRANSITION OF DNA HAIRPINS WITH A d(CG)$_3$ STEM.

M. Amaratunga, P. Pancoska, T. M. Paner and A. S. Benight. Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680.

Circular Dichroism (CD) spectra of the DNA hairpins formed from the oligomer sequences d[(CG)$_3$X$_4$(CG)$_3$] (X=A,T,G,C) collected as a function of sodium perchlorate concentration [NaClO$_4$] are reported. Over the range from 0 to 4.0 M [NaClO$_4$], the CD spectra invert in a manner characteristic of the B to Z transition. Factor analysis is employed to determine the least number of basis spectra required to fit the measured spectra of each hairpin over the entire salt range examined. In every case, linear combinations of only two subspectra fit the experimental spectra of the hairpins with greater than 98% accuracy, indicating the spectrally monitored structural transitions are two-state. B-Z transition curves are constructed from the relative weights of the individual subspectra. Analysis of the transitions in terms of a simple two-state equilibrium model yields an evaluation of the B-Z transition free-energy as a function of [NaClO$_4$] and loop sequence.

W-Pos150

EFFECTS OF 5′ DANGLING SINGLE STRAND ENDS ON THE THERMODYNAMIC STABILITY OF DNA HAIRPINS. M. J. Doktycz, T. M. Paner, M. Amaratunga and A. S. Benight. Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680.

Optical melting curves of 17 DNA hairpins containing the common six base-pair duplex stem sequence 5′GGATAC3′ and a T_4 single strand loop on the 3′ end were measured in 100 mM NaCl. 16 of the hairpins contain a four base single strand 5′ end comprised of a repeating doublet of one of the 16 possible 5′-3′ nearest-neighbor nucleotide stacks in DNA. The 17^{th} oligomer is a blunt-ended hairpin and serves as the internal reference. Results demonstrate *all* dangling ends induce increases in transition temperature, some as great as 3.8°C above the blunt-ended reference. Correlations between dangling-end sequence and hairpin stability are observed. Quantitative estimates on the upper limits of the thermodynamic transition parameters are obtained by analyzing experimental melting curves in terms of the exact statistical thermodynamic model of DNA hairpin melting. Estimates of the average contribution to duplex stability of each of the 16 possible nearest-neighbor 5′ to 3′ single strand stacks in DNA will be presented.

W-Pos152

STRUCTURAL FEATURES OF HIGH AFFINITY CARCINOGEN BINDING SITES. Stephen A. Winkle, Dept. of Chemistry, Florida International University, W. John Layton, Dept. of Chemistry, University of Kentucky, & Richard D. Sheardy, Dept. of Chemistry, Seton Hall University.

Previously, we have reported that the carcinogen N-acetoxy-N-acetyl-2-aminofluorene preferentially binds to a small group of sequences on a variety of DNAs, i.e. PBr322, phiX174, SV40 [S.A. Winkle, et al, BIOPHYSICAL J. 55, 242a (1989)]. Using 1-D and 2-D NMR techniques and enzymatic assays, we have examined the structure of an oligomer which is representative of these binding sites:

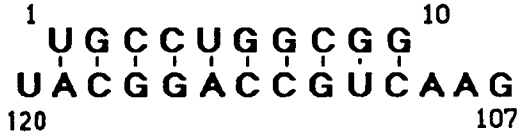
5′-GTCAGCTCTTG*CTGCG
3′-CAGTCGAGAACGACGC

These studies, conducted in the absence of bound carcinogen, suggest that near the carcinogen binding site (G* above), the oligomer appears to be more temperature-labile. Inhibition assays with lambda exonuclease, exonuclease III, and the restriction nuclease Alu I suggest that, near the binding site, the conformation may not be exactly a "traditional" B-type structure - in the absence of bound AAAF. Suggestions regarding the structure of the binding site will be discussed.

W-Pos151

AN NMR STUDY OF HELIX I OF E. COLI 5S RIBOSOMAL RNA. S. A. White and P. B. Moore, Department of Chemistry, Yale University, New Haven, CT 06511.

Structural determination of biologically important RNA sequences by two-dimensional NMR techniques has only recently become practical with the development of methods for large scale RNA production. Helix I of E. coli 5S ribosomal RNA has been produced by nucleolytic cleavage of overproduced 5S RNA from the rrnB operon. The imino proton spectrum is a subset of the NMR spectrum for a larger 5S fragment which contains Helices I, IV, and V. The imino proton assignments were verified using one-dimensional NOE experiments. As expected, the G-U base pair has two strong imino resonances at 296K. Two-dimensional NOESY experiments reconfirmed and extended these assignments. Further assignments in the aromatic and sugar regions will be presented.



HELIX I

W-Pos153

DUPLEXES AND TRIPLEXES OF OLIGODEOXYRIBONUCLEOSIDE METHYLPHOSPHONATES. D.E. Callahan, T.L. Trapane, P.S. Miller, P. O.P. Ts'o, & L.-S. Kan, Div. Biophys., S.H. P.H., Johns Hopkins Univ., Baltimore, MD

Nucleic acid analogs containing nonionic methylphosphonate internucleoside linkages (NpN) can enter cells and may be able to regulate gene expression by sequence-specific triplex formation with DNA or duplex formation with RNA. Using 16-mers as a model system, we have shown $d(CT)_8$ and $d(AG)_8$ form triplex at pH 5.2 with the same CD spectrum as that of poly[d(CT)·d(AG)·d(C$^+$T)] at pH 5.5. A similar CD spectrum for a 1:2 mixture of $d(AG)_8$ and $dCpTp(CpT)_7$ was obtained at pH 4.1, indicating triplex formation. A decrease of ethidium bromide flourescence enhancement (indicating the presence of a third strand in the duplex major groove) supports the CD conclusions. CD and UV melting curve studies at pH 7.0 indicate that the conformations of duplexes containing $dCpTp(CpT)_7$ are different and the Tm's are lower (40°C) than duplex containing no methylphosphonate (50°C) or duplex containing only the purine methylphosphonate $dApGp(ApG)_7$ (46°C). These data support a possible steric hindrance between the T-methyl and the phosphonate methyl group in the duplexes. (supported in part by DOE and NCI)

W-Pos154

CHARACTERIZATION OF AFLATOXIN B_1-OLIGODEOXYNUCLEOTIDE ADDUCTS BY ^1H NMR

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Aflatoxin B_1-oligodeoxynucleotide adducts were constructed by reaction of aflatoxin B_1-8,9-epoxide with $d(ATCGAT)_2$ and $d(ATGCAT)_2$. Whereas $d(ATCGAT)_2$ reacts with one equivalent of epoxide to form a non-symmetrical mono adduct, $d(ATGCAT)_2$ reacts with two equivalents of epoxide to form a symmetrical bis adduct. ^1H NMR spectral assignments of each adduct have been derived. The proton at C(8) of the modified guanine exchanges with solvent and is observed at 9.75 ppm. For both modified oligodeoxynucleotides, six resonances arising from N-H--N hydrogen bonds are observed between 12-14 ppm. ^1H NOE measurements reveal that in both instances, the aflatoxin moiety is intercalated over the 5' face of the modified guanine. NOE connectivities are observed between each oligodeoxynucleotide and aflatoxin B_1. In addition, several oligodeoxynucleotide NOEs are missing after adduct formation. The difference in reaction stoichiometry for the two oligodeoxynucleotides is consistent with intercalation of the aflatoxin moiety in the adduct. The presence of the aflatoxin moiety above the 5'-face of guanine in modified $d(ATCGAT)_2$ prevents binding of a second molecule of aflatoxin B_1-8,9-epoxide, whereas two sites can be occupied by aflatoxin B_1-8,9-epoxide in $d(ATGCAT)_2$. Supported by ES-03755 and ES-00267.

W-Pos156

VIBRONIC SPECTROSCOPY OF Gd^{3+} BOUND TO DNA.

I E T Iben+, *R B Macgregor, Jr.*, *M Stavola*, *J M Friedman*+ (AT&T Bell Labs, Murray Hill, NJ & +New York University, Chemistry) & *R Austin* (Princeton University, Physics).

Gd^{3+} luminescence spectra ($6P_{7/2}$ -> $6S_{7/2}$) exhibit vibronic side bands that reflect the vibrational energy levels of the molecules to which it coordinates. We have used this property to study the IR spectra of poly(dA)• poly(dT) and poly[d(A-T)] isolated to the coordination sites of Gd^{3+}, yielding information on DNA-ion interactions. The spectra exhibit vibronic peaks that we attribute to carbonyls, phosphates and water.

The lifetime, τ_f of the $6P_{7/2}$ state remains constant from 10K up to a temperature T_g, where it begins decreasing. For Gd^{3+} coordinated to water, T_g is ~180K, and ~200K when coordinated to EDTA in water. Below T_g, the $6P_{7/2}$ state is Stark-split into four levels. The absolute energy of and the separation between the Stark-split states are influenced by the molecular environment of the Gd^{3+}. Below T_g the emission is excitation-wavelength dependent; this dependence is not found at temperatures above T_g. We associate T_g with a glass transition of the molecule-solvent system.

W-Pos155

HYDRALAZINE-DNA INTERACTION INVOLVES LEFT-HANDED Z-DNA FORMATION.

T.J. Thomas, L.L. Ong and U.B. Gunnia, Department of Medicine, UMDNJ-RWJ Medical School, New Brunswick, NJ 08903.

Hydralazine (Hy) is a member of the lupus-inducing drugs. These drugs are used in the treatment of various illnesses including hypertension and heart diseases, and produce a lupus-like syndrome in the recipients. The presence of anti-DNA and anti-histone antibodies is a hallmark of this disease. In order to understand the mechanism of production of antinuclear antibodies in drug-induced lupus, we studied the interaction of hydralazine with poly(dG-m^5dC).poly(dG-m^5dC) (I) and recombinant plasmids using spectroscopic techniques, enzyme immunoassay (EIA) and gel electrophoresis. Using CD spectroscopy and EIA with a monoclonal anti-Z-DNA antibody (Z22), we found that Hy provoked the Z-DNA form of I. Gel electrophoretic analysis of recombinant plasmids further showed Z-DNA formation in $(dA\text{-}dC)_n.(dG\text{-}dT)_n$ and $(dG\text{-}dC)_n.(dG\text{-}dC)_n$ inserts. A control plasmid devoid of these Z-DNA forming inserts showed no binding to Z22. In contrast to the poor immunogenicity of B-DNA, Z-DNA is immunogenic and produces anti-Z-DNA antibodies in experimental animals. Therefore, a possible mechanism of action of hydralazine might involve the interaction of the drug with labile DNA sequences to provoke the immunogenic Z-DNA from.

W-Pos157

THE X-RAY STRUCTURAL ANALYSIS OF TWO DNA OLIGONUCLEOTIDES: $d[CGC(O^6Me)GCG]_2$ AND $d[CGTGAATTCACG]_2$

Stephan L. Ginell, N. Narendra, Roger Jones and Helen M. Berman, Department of Chemistry, Rutgers University, New Brunswick, NJ and Irina M. Russu, Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, Connecticut (Intro. by A. Joshua Wand)

Two DNA oligonucleotide crystal structures have been determined at -90°C. The first, $d[CGC(O^6Me)GCG]_2$, contains an (O^6Me) guanine and crystallizes in the Z-DNA conformation. Although it was expected to have wobble base pairing, in fact it displays the Watson Crick orientation. The second, $d[CGTGAATTCACG]_2$, contains the Eco R1 recognition and cleavage site but a different flanking sequence than the dodecamer structure determined by Drew and Dickerson. Details of the experimental procedures as well as a description of the conformation, packing and solvent structure will be presented.

This work has been supported by grants from the NIH and NSF.

W-Pos158

A POLARIZED PHOTOBLEACHING STUDY OF CHROMATIN REORIENTATION IN NUCLEI. Paul R. Selvin (1,2), Bethe A. Scalettar (1), John P. Langmore (4), Daniel Axelrod (4), Melvin P. Klein (1), & John E. Hearst (1,3). (1) Chemical Biodynamics Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720; (2) Dept. of Physics (3) Dept. of Chemistry, U.C. Berkeley, Berkeley, CA 94720; and (4) Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109.

Polarized fluorescence recovery after photobleaching was used to monitor the micro- and millisecond reorientational dynamics of chromatin in mudpuppy nuclei as a function of salt concentration. Under conditions in which the 30 nm chromatin fibers are intact and aggregated (physiological level monovalent salt with divalent cations) the chromatin was immobile. Removal of the divalent cations to disperse the fibers led to a millisecond reorientational relaxation. Subsequent dilution of the monovalent salt, which is known to cause the 30 nm fiber to decondense into a beads-on-a-string structure, caused a dramatic increase in chromatin flexibility. The data indicate that the structural and dynamic transitions of chromatin occur gradually and that the high local concentrations of chromatin that are found *in vivo* are a major determinant of chromatin dynamics.

This work was supported by NIH grants GM41911, NS14565 & GM27937, NSF grants DMB8805296 & DIR8706052, DOE grant DE AC03-76SF00098, and an Alexander Hollaender Fellowship.

W-Pos160

SCANNING TUNNELING MICROGRAPHS OF SINGLE-STRANDED NUCLEIC ACIDS

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The scanning tunneling microscope has the potential to image biological macromolecules with atomic detail, but recent images of DNA have not realized such resolution. A poor understanding of the imaging mechanisms is partly to blame, but also elusive is a mechanically stable sample preparation. We hypothesized that single-stranded DNA, with exposed, uncharged bases, would adsorb more stably onto graphite and be less susceptible to perturbation by the tip. The coincident lateral spreading of the molecule also might make the bases accessible for imaging. By depositing polydeoxyadenylate, we observed molecules aligned in parallel with their bases lying flat and the charged phosphodiester backbone raised upward, in a manner consistent with the hydrophobicity of graphite. The images correspond well with a molecular model, that also indicates a hydrogen bond that could stabilize the parallel alignment. These micrographs demonstrate the utility of the scanning tunneling microscope for structural studies of nucleic acids and provide evidence that it could be used to sequence DNA.

W-Pos159

A POLARIZED PHOTOBLEACHING STUDY OF DNA REORIENTATION IN AGAROSE GELS. Bethe A. Scalettar (1), Paul R. Selvin (1,2), Daniel Axelrod (4), Melvin P. Klein (1), & John E. Hearst (1,3). (1) Chemical Biodynamics Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720; (2) Dept. of Physics & (3) Dept. of Chemistry, U.C. Berkeley, Berkeley, CA 94720; and (4) Dept. of Physics, University of Michigan, Ann Arbor, MI 48109.

Polarized fluorescence recovery after photobleaching has been used to study the internal dynamics of relatively long DNA molecules embedded in agarose gels. The data indicate that, even in very congested gels, rapid internal relaxation of DNA is largely unhindered; however, interactions with gel matrices apparently do perturb the larger amplitude, more slowly (μsec and msec) relaxing internal motions of large DNA's. The relationship between this work and recent studies which indicate that internal motions of DNA play an important role in the separation achieved with pulsed-field gel electrophoresis techniques is discussed. The polarized photobleaching technique is also analyzed in some detail. In particular, it is shown that "reversible" photobleaching phenomena are probably related to depletion of the ground state by intersystem crossing to the triplet state.

This work was supported by NIH grants #GM41911 & #NS14565, NSF grant #DMB8805296, DOE grant #DE AC03-76SF00098, and an Alexander Hollaender Fellowship.

W-Pos161

A LYOTROPIC SERIES FOR THE B-TO-Z TRANSITION IN DNA. N.B. McDonnell and R.S. Preisler, Dept. of Chemistry, Towson State University, Towson, MD 21204.

The effect of salt anion size on the B-to-Z transition midpoint in poly[d(G-C)] and poly[d(G-m^5C)] was investigated using circular dichroism. With an alkali metal as cation an increase in anion size from fluoride to iodide or perchlorate facilitates the transition. The larger anions in this series have a chaotropic or structure-breaking effect on the aqueous solvent. On the other hand, effectiveness increases with structure-forming tendency among ions with a hydrophobic moiety (carboxylate anions and tetraalkylammonium cations). A simple model system may explain these diverse trends. Guanosine residues are more exposed to solvent in Z-DNA than in B-DNA. Salts may influence the B-Z equilibrium according to their effect on the activity coefficient of guanosine in DNA. To test this hypothesis we are measuring the solubility of free guanosine in solutions of various salts. Preliminary results show a correlation between solubilization of guanosine and efficiency in driving the B-to-Z transition. Supported by grants from the Faculty Research Committee, TSU.

W-Pos162

SOLUTION STRUCTURES OF LAMBDA OPERATORS O_R3 AND O_L1 PROBED BY RAMAN SPECTROSCOPY. J. M. Benevides & G. J. Thomas, Jr. Div. Cell Biol. & Biophysics, School of Basic Life Sciences, University of Missouri - Kansas City, Kansas City, MO 64110

We employed Raman spectroscopy to compare solution secondary structures of the bacteriophage lambda operators O_R3 [d(5'TATCACCGCAAGGGATA)] and O_L1 [d(5'A-TACCACTGGCGGTGATA-T)], each paired with its Watson-Crick complement, at conditions favoring duplex formation. Raman bands which serve as markers of phosphodiester conformation indicate for each operator a right-handed B-DNA structure, similar to that formed by DNA of GC composition and characterized by a relatively wide minor groove. Nucleoside conformations, which are also marked by Raman bands, are of the C2'endo/anti type. The Raman spectra also reveal significant differences between O_L1 and O_R3 which may be of importance for repressor recognition. Spectra/structure correlations indicate that torsion angles (α,β,γ) of 2 residues per strand are all-trans in O_L1, rather than the more conventional (g^-,t,g^+) configuration which is observed in O_R3. [NIH AI18758]

W-Pos163

EXPERIMENTAL AND COMPUTATIONAL EVALUATION OF 16 DNA WEDGE ANGLES, A. Bolshoy[1], P. McNamara[2], R.E. Harrington[2] and E.N. Trifonov[1]. (1) Department of Polymer Research, the Weizmann Institute of Science, Rehovot, 76100 ISRAEL (2) Department of Biochemistry, University of Nevada Reno, Reno, NV 89557 USA.

DNA curvature as reflected in its anomalous behavior in polyacrylamide gels, is a function of sequentially added wedge vectors corresponding to 10 different stacks of base pairs in DNA. All dinucleotide wedges have roll and tilt components except self-complementary stacks with rolls only, the total being 16 wedge angles. 50 experimental measurements of anomaly coefficients for synthetic DNA fragments of various sequences are expressed as a system of 50 non-linear equations for 16 unknowns, and the unique solution of the system is found by the conjugate-gradient method. The solution obtained satisfies all the experimental data with average misfit close to 1 standard deviation of the measurements.

W-Pos164

THE SEQUENCE PREFERENCES OF DNA BINDING BY ANTI-(-)- AND ANTI-(+)-BENZO[a]PYRENE-7,8-DIOL-9,10-EPOXIDE. **Glenn A. Marsch and Randolph L. Rill, Dept. of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306.**

The sequence preferences of *alkali-labile* adduct formation by the carcinogen anti-(-)-benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) were examined on over 1200 bases by techniques analogous to chemical DNA sequencing. All bases yielded labile products, the reactivities being in the order G>A,C>>T. Base reactions were non-random and depended on sequence context. The most reactive triplets were AGG, CGG, and TGN (N = any base but A, most reactive base underlined). RGR sequences were less reactive than average, *excepting* AGG. (Abbreviations: R = purine, Y = pyrimidine.) RGY sequences were usually less reactive than average; none were more reactive than average. YGG sequences were generally much more reactive than average, while YGA sequences exhibited varied reactivities. TGY sequences were more reactive than average. Anti-(+)-BPDE also reacted preferentially at AGG and CGG sequences, but not TGN sequences. Supported by a grant from DOE.

W-Pos165

SEQUENCE SPECIFIC CONFORMATIONAL VARIATION IN DNA-ECHINOMYCIN COMPLEXES Dara Gilbert and Juli Feigon, Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California at Los Angeles, CA 90024

Echinomycin binds to DNA as a bis-intercalator at CpG steps. The conformation of the DNA adjacent to the binding site changes dramatically as a result of drug binding. We have shown, based on high field NMR studies, that in the complex $[d(ACGTACGT)]_2$ - 2 echinomycin the terminal AT base pairs are Hoogsteen base paired and that the central AT base pairs are Hoogsteen base paired at low temperature but are in an alternate conformation at higher temperature (Gilbert *et al* (1989) PNAS **86**, 3006-3010). We are studying several other complexes in order to elucidate the sequence and positional requirements for structural changes in the DNA duplex. Echinomycin binds to the duplex TCGATCGA by bis-intercalation. No Hoogsteen basepairs are formed; nor are the central AT base pairs destabilized as a result of drug binding. The drug also intercalates into the duplex of the sequence GGAACGTTCC There are no Hoogsteen base pairs formed when echinomycin binds at central CpG step yet all four AT base pairs adjacent to the binding site are less stable than those in the free DNA. A comparison of the three complexes will be presented.

W-Pos166

Two-Dimensional NMR Studies of a Carcinogen-Modified Oligomer.

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Department of Chemistry University of Rochester
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An N-2-acetylaminofluorene (AAF) modified deoxyoligonucleotide duplex, d(C1-C2-A3-C4-^{AAF}G5-C6-A7-C8-C9)·d(G10-G11-T12-G13-C14-G15-T16-G17-G18), was studied by one- and two-dimensional NMR spectroscopy. The three base pairs on each end of the duplex exhibit nOe's characteristic of right-handed B-form DNA. The major structural distortion is associated with G5 adopting the *syn* conformation, as indicated by nOe's between the G5 imino proton and the A3-H3′ and A3-H2′/H2′′ protons, as well as nOe's between the fluorene-1 proton of AAF and the G5-H1′ or C6-H1′ proton. This G5 orientation places the carcinogen in the minor groove. With G5 being *syn*, we find no evidence for hydrogen bonding of G5 and C14. Another structural anomaly is that there are no observable nOe's between the A3 sugar protons and the C4 base protons, but there is a rather strong nOe between A3-H2′/H2′′ and C4-H1′. Structural distortion of the unmodified strand is evident from the fact that many of the internucleotide nOe's between T12, G13, C14, and G15 are weaker than would be expected for B-DNA, while the nOe's between G13-H3′and C14-H6 and between C14-H4' and G15-H8 are unusually strong. Chemical exchange appears to arise from different conformations of the bonded AAF moiety. There does not appear to be any structural changes in the pH range of 6.0 to 8.5. Energy minimization studies, in collaboration with S. Broyde and B. Hingerty, are in progress to develop a model incorporating the structural features determined by NMR.
Supported by NCI grant CA-35251.

W-Pos168

CRYSTAL FIELD EFFECTS ON TRANSITION MOMENTS IN METHYLATED ADENINES N. Sreerama and R.W. Woody (Dept. of Biochem., Colorado State Univ., Ft. Collins, CO-80523) and P.R. Callis (Dept. of Chem., Montana State Univ., Bozeman, MT-59717)

The transition moment directions of nucleic acid bases are important in understanding the optical properties of nucleic acids. There are discrepancies between the experimental data from polarized spectra of single crystals and semiempirical MO results, which probably originate in the electrostatic environment in the crystal. Such effects have been treated by incorporating the time-average field into the Hamiltonian. We have applied this method to two adenine chromophores, 9-methyladenine and N^6-methyladenine. The electrostatic fields and potentials were calculated by using ground state INDO/S wavefunctions for the surrounding molecules. The strongest transitions in 9-MeAde and N^6-MeAde crystals are similar in direction and close to those obtained experimentally. The weak transitions differ by ~70° and are predicted to have higher energies than the major bands, in contrast to experiment (L.B. Clark, J. Phys. Chem., *93*, 5345, 1989). (Supported by USPHS grants GM22994 & GM31824).

W-Pos167

CRYSTAL FIELD EFFECTS ON TRANSITION MOMENTS IN 9-ETHYLGUANINE D. Theiste and P.R. Callis (Department of Chemistry, Montana State University, Bozeman, MT 59717) and R.W. Woody (Department of Biochemistry, Colorado State University, Fort Collins, CO 80525).

Electrostatic interactions may be largely responsible for discrepancies between transition dipole moment directions observed in single-crystal absorption or reflection spectra and those calculated by semiempirical MO theory. In 9-ethylguanine the discrepancy is severe: INDO/S and experiment differ by 40° and 48°, respectively for the first two $\pi\pi^*$ transitions. The Ridley-Zerner INDO/S program has been modified to include electrostatic effects. All surrounding molecules within a radius of 20 Å are considered. To account for induced dipole moments in the crystal, an iterative procedure has been used. Since INDO/S overestimates the ground-state dipole moment of 9-methylguanine, we have also carried out calculations using charges scaled by the ratio of the ab initio and INDO/S dipole moments. The effects are significant and agreement with experiment is markedly improved. (Supported by NIH grants GM22994 to RWW and GM31824 to PRC).

W-Pos169

LINEAR DICHROISM AND GEL ELECTROPHORESIS STUDIES OF THE UNWINDING OF SUPERCOILED DNA BY DIFFERENT STEREOISOMERS OF BENZO[*a*]PYRENE DIOL EPOXIDE.

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The effects of binding of the tumorigenic (+)- and non-tumorigenic (-)-enantiomers of BPDE (*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene) on the gel electrophoresis and flow linear dichroism characteristics of supercoiled PIBI 30 and ϕX 174, were studied. Flow linear dichroism spectra indicate strikingly different adduct conformations for covalently bound (+) and (-)-BPDE. Only the tumorigenic (+)-isomer produces a significant decrease in the electrophoretic mobility of PIBI30 and ϕX 174; this effect depends on the level of binding of BPDE, and may be due to both unwinding and/or the formation of flexible joints or bends at the adduct binding sites.
Supported by NIH-NCI and DOE.

W-Pos170

DIFFERENCES IN CHARACTERISTICS OF SUPERCOILED AND LINEAR DNA DUE TO COVALENT BINDING OF 5- AND 6-METHYL CHRYSENE DIOL EPOXIDE DERIVATIVES.

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Linear dichroism and agarose gel electrophoresis techniques were used to study and compare the effects of (+)-5-MeCDE and (+)-6-MeCDE covalent binding to supercoiled PIBI 30 DNA and a linearized DNA fragment (Eco R1 digest of PIBI 30). In contrast to the much less active (+)-6-MCDE, the highly tumorigenic (+)-5 derivative gives rise to remarkably large changes in the electrophoretic mobilities of PIBI 30 and decreased flow linear dichroism signals of the linear DNA. These and other studies suggest that highly tumorigenic and mutagenic polycyclic aromatic diol epoxides profoundly influence the characteristics of DNA (gel electrophoretic mobilities, degree of alignment in a hydrodynamic flow gradient), whereas their less active/inactive positional isomers or stereoisomers do not.

Supported by NIH-NCI and DOE.

W-Pos171

MOLECULAR DYNAMICS SIMULATIONS OF OLIGOMER GUANINE N^2 - (+) AND (-) ANTI BENZO(a)PYRENE DIOL EPOXIDE ADDUCTS WITH SOLVENT AND SALT.

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Molecular dynamics simulations (MDS) have been carried out on (+) and (-) anti BPDE modified duplex heptamers with explicit solvent and salt using AMBER/NEWTON (3.1). Starting conformations were energy minimized structures generated from extensive conformational searches for BPDE modified deoxydinucleoside monophosphates or duplex trimers built into larger oligomers with the program DUPLEX. Three important structural types were found with DUPLEX and investigated in the MDS for each enantiomer: pyrenyl residue in (i) major groove; (ii) minor groove; and (iii) approximately coplanar with base planes. The MDS effectuated important rearrangements of some conformers within 60 ps which did not occur in control MDS without explicit solvated. Computed structures will be compared with experimental results.

Supported by NIH, DOE and NSF.

W-Pos172

PROBING THE HYDRATION OF THE MINOR GROOVE OF NUCLEIC ACIDS BY VOLUME AND HEAT CHANGES

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Water plays a fundamental role in the stabilization of the secondary structure of nucleic acids. Despite much experimental and theoretical work, we know very little today about sequence hydration effects in these molecules. We address this problem by a combination of densimetric and calorimetric techniques to measure directly the ΔV^o, ΔH^o and K_b parameters for the association of drugs to polynucleotides. Previously, we have reported on the binding of netropsin (a minor-groove ligand) to A·T DNA polymers and concluded that poly(dA)·poly(dT) was much more hydrated than poly(dAT)·poly(dAT). In the present work we have extended these studies to include poly (dGC)·poly(dGC) and poly(rA)·poly(dT), as well as with the ligand distamycin A. With both ligands the order of hydration is: poly(dA)·poly(dT) > poly(dAT)·poly(dAT) > poly(rA)·poly(dT) > poly(dGC)·poly(dGC). In addition the ΔV results correlate conveniently with the ΔS values obtained from titration calorimetry experiments.

Funded by NIH Grants GM-42223 and GM-34938.

W-Pos173

UV PHOTOELECTRON AND *AB INITIO* QUANTUM MECHANICAL EVALUATION OF IONIZATION POTENTIALS OF NUCLEOTIDES

Kenzabu Tasaki, Xu Yang, Shigeyuki Urano, Sharon Fetzer, and Pierre R. LeBreton, Department of Chemistry , University of Illinois at Chicago, Chicago, Illinois 60680

He(I) UV photoelectron spectroscopy and SCF *ab initio* molecular orbital calculations with the 4-31G basis set have been employed to characterize the valence electronic structures of neutral and anionic 2′-deoxycytidine-5′-phosphate (5′-dCMP). Both the neutral and the anion contain 160 electrons. The results of SCF calculations at the 4-31G level on neutral 5′-dCMP demonstrate that, in the nucleotide, the electron distributions for the eleven highest occupied orbitals are similar to orbitals appearing in 1-methylcytosine, 2′-deoxyribose ,and methylphosphate. A similar localization of upper occupied orbitals on either the base, sugar , or phosphate group is observed in results for anionic 5′-dCMP.

The results have been employed to interpret alkylation patterns for DNA and RNA methylation and ethylation reactions.

W-Pos174

A COMPARISON OF DNA INFLUENCES ON THE REACTIVITIES OF (±) *trans*-7,8-DIHYDROXY-*anti*-9,10-EPOXY-7,8,9,10-TETRAHYDRO-BENZO(a)PYRENE (BPDE), BENZO(a)PYRENE-4,5-OXIDE (BPO), AND BENZ(a)ANTHRACENE-5,6-OXIDE (BAO)

Shigeyuki Urano, Harry L. Price, Sharon M. Fetzer, Kenzabu Tasaki, Anita Briedis, Ann Milliman and Pierre R. LeBreton, Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680

Pseudo first-order reaction rate constants have been measured for reactions of BPDE, BPO and BAO. In 10^{-3} M sodium cacodylate, the rate constants (in sec^{-1}) increase in the order: BPO $(3.8\pm0.1)x10^{-6}$ < BAO $(5.7\pm2.6)x10^{-5}$ < BPDE $(7.2\pm1.0)x10^{-4}$.

These results have been compared with pseudo first-order rate constants for reactions carried out in calf thymus DNA. Here the rate constants increase in the order: BAO $(2.8 \pm 0.1)x10^{-3}$ < BPO $(1.2 \pm 0.2)x10^{-2}$ < BPDE $\approx 1.0x10^{-1}$.

The ordering of rate constants observed when reactions of BPDE, BPO and BAO are measured in DNA, do not correlate with the ordering of rate constants measured in buffer alone. They do correlate with association constants for the intercalative binding of BPDE, BPO and BAO into DNA.

W-Pos176

SCANNING TUNNELING MICROSCOPY OF COATED AND UNCOATED *E. COLI* RNA POLYMERASE AND λ DNA

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Both metal coated and uncoated *E coli* RNA polymerase bound to λ DNA have been imaged by STM. The metal coated samples show RNA polymerase molecules bound to the DNA together with free polymerase and free DNA at a resolution of about 40Å. These samples showed that the RNA polymerase molecules were extensively bound to the DNA molecules in some areas. Experiments with uncoated samples were also conducted. The uncoated experiments promise much higher resolution than the coated experiments since the metal coating can obscure detail that might otherwise be seen. Under identical deposition conditions, except for the metal coating, the uncoated samples showed only a few free polymerase molecules weakly bound to the surface. It is apparent from these results that even though molecules can be seen on occasion, they are either not securely attached to the surface or too insulating to be seen by the STM in a reliable way. These problems may be overcome by controlling the humidity, reducing the presence of oxygen, and by modifying the substrate to bind the sample molecules more firmly to the surface.

W-Pos175

BINDING OF MERCURIC ION TO POLY(dA):POLY(dT).

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Poly(dA):poly(dT) exhibits striking absorption spectral alterations and induces a unique intense positive CD band at 296 nm during the spectral titrations with $HgCl_2$ at pH 9.2. Spectral comparison with 2-component single-strand titrations suggests a binding model in which the mercuric ions preferentially bind to this homopolymer initially via cross-linkings to N3 of two thymines and cause the eventual strand separation of the duplex, with further binding to poly(dA) at higher mercuric concentrations. In line with the proposed model, analyses of the binding isotherms of single-strand titrations suggest that Hg(II) binds to poly(dT) much more strongly than to poly(dA), with saturation binding densities estimated to be 1 Hg per 2 bases and 1 Hg per base, respectively. Binding to poly(dA), however, is extremely cooperative. Effects of base sequence and stacking are investigated by binding studies with dinucleoside monophosphates and mononucleosides. (Research supported by USPHS Grant CA-42682 and by a subproject of MBRS Grant S06RR0892).

W-Pos177

SEQUENCE DEPENDENT INFRARED VIBRATIONAL CD IN B-FORM MODEL DNA

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Infrared (Vibrational) CD (VCD) has been observed for the DNA models $(CG)_5$, poly(dG-dC).poly(dG-dC), poly(dG).poly(dC), poly(dA-dT).poly(dA-dT), and poly(dA).poly(dT) in the B-conformation in aqueous solution. We find that C-G polymers give quite different spectra than A-T polymers. In addition, the VCD spectra show different spectra for poly(dG).poly(dC) and poly(dG-dC).poly(dG-dC). The observed results are quantitatively interpreted in terms of the carbonyl stretching vibrational states.

We find, in general, excellent agreement between observed and theoretical spectra. Thus it appears that VCD can be used as a novel spectroscopic technique to sample local effects in DNA solution conformation.

Grant Support: NIH GM 28619

W-Pos178

STRUCTURAL SPECIFICITY OF POLYAMINES IN B→Z TRANSITION OF RECOMBINANT PLASMIDS. U. B. Gunnia, T. Thomas and T.J. Thomas, Departments of Medicine and Env. & Comm. Medicine, UMDNJ-RWJ Medical School, New Brunswick, NJ 08903.

Polyamines --putrescine^{2+} (put) , spermidine^{3+} (spd), and spermine^{4+} (spm)-- are small organic cations found in all living cells. They provoke the left-handed Z-DNA conformation in certain polynucleotides. To examine the role of polyamines in the conformation of small inserts of $(dA\text{-}dC)_n.(dG\text{-}dT)_n$ (I) and $(dG\text{-}dC)_n.(dG\text{-}dC)_n$ (II) blocks in plasmid DNAs, we conducted an enzyme immunoassay with a monoclonal anti-Z-DNA antibody (Z22) on 3 recombinant plasmids --pDPL6, control; pDHf2 (I; n=12); and pDHg16 (II; n=12)-- in the presence and absence of polyamines. Spermidine and spermine converted I and II to the Z-DNA from. The B→Z midpoint concentrations were: I, spd 15 μM and spm 10 μM; II, spd 80 μM and spm 8 μM. Agarose gel electrophoresis confirmed complex formation between plasmid DNAs and Z22. In contrast, there was no conformational alteration in the control plasmids by spd or spm. Using a series of spermidine homologs, we also found that spd was the most efficacious triamine to provoke Z-DNA form of I and II. These results suggest that a possible mechanism of the gene regulatory effects of polyamines might involve the induction of Z-DNA form in labile DNA segments.

W-Pos179

CONFORMATIONAL DYNAMICS OF POLYNUCLEOTIDES IN THE PRESENCE OF RHODIUM COMPLEXES. T.J. Thomas and Thresia Thomas, Departments of Medicine and Env. & Community Medicine, UMDNJ-RWJ Medical School, New Brunswick, NJ 08903.

We studied the effects of hexammine and tris(ethylene diamine) complexes of rhodium on the conformation of poly(dG-dC).poly(dG-dC) (I) and poly(dG-m^{5}dC).poly(dG-m^{5}dC) (II) using spectroscopic techniques and an enzyme immunoassay (EIA). CD measurements showed that $Rh(NH_3)_6^{3+}$ provoked a B-DNA→Z-DNA→Ψ-DNA conformational transition in I. Using the EIA technique with a monoclonal anti-Z-DNA antibody, we found that the left-handedness of the polynucleotide was maintained in the Ψ-DNA form. We also compared the efficacy of $Rh(NH_3)_6^{3+}$ and $Rh(en)_3^{3+}$ to provoke the Z-DNA conformation of I and II. The concentrations of $Rh(NH_3)_6^{3+}$ and $Rh(en)_3^{3+}$ at the midpoint B-DNA → Z-DNA transition of I were 48 ± 2 and 238 ± 2 μM, respectively. The Ψ-DNA form of I was stabilized at 500 μM $Rh(NH_3)_6^{3+}$. With II, both counterions provoked the Z-DNA form at approximately 5 μM and stabilized the polynucleotide in this form up to 1000 μM concentration. These results show that trivalent complexes of Rh have a profound influence on the conformation of poly(dG-dC).poly(dG-dC) and its methylated derivative.

W-Pos180

PARTIAL REACTIVATION OF 3-HYDROXYBUTYRATE DEHYDROGENASE BY PHOSPHATIDYLETHANOLAMINE (PE) AND N-METHYLATED PE ANALOGUES.

J. Oliver McIntyre, Qiu-chen Cheng, Hansjorg Eibl and Sidney Fleischer. Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235 and Max-Planck Institute, Gottingen, FRG.

BDH is a lipid-requiring enzyme purified from the mitochondrial inner membrane. ApoBDH is devoid of phospholipid (PL) and inactive but can be reactivated by insertion into PL vesicles containing phosphatidylcholine (PC). PC is required for optimal activity and proper nucleotide binding to BDH [Rudy *et al.*, Biochemistry 28, 5354 (1989)]. We now find that partial activity can be restored to apoBDH by PE or N-methylated PE analogues, i.e., N-methyl-PE (MonoMePE) and N,N-dimethyl-PE (DiMePE) in phospholipid vesicles codispersed with diphosphatidylglycerol (DPG). For the different BDH-PL complexes, the BDH activities [30°C, 20 mM R,S-3-hydroxybutyrate (BOH), 10 mM NAD^+] were 50 (PC), 30 (DiMePE), 3 (MonoMePE) and 0.4 (PE) μmol NAD^+ reduced·min^{-1}·mg^{-1}. PC, in contrast to PE, stabilizes BDH activity at 37°C. Kinetic analysis (30°C) yields apparent K_m values for BDH in PC vs PE of: 0.1 vs 35 mM for NAD^+; 1.4 vs ~250 mM for R-BOH; 0.05 vs 1.6 mM for NADH; and 1.4 vs 8.0 mM for acetoacetate. We conclude that the allosteric activation of BDH improves with sequential N-methylation of PE; optimal activation of BDH by PC is referable to enhanced coenzyme binding (10- to 100-fold lower K_m).

[Supported in part by NIH DK 14632.]

W-Pos181

MODULATION OF 3-HYDROXYBUTYRATE DEHYDROGENASE (BDH) BY SUBSTRATE ANALOGUES.

Qiu-chen Cheng, J. Oliver McIntyre, Thomas M. Duncan, Wolfgang Trommer and Sidney Fleischer. Department of Molecular Biology, Vanderbilt University, Nashville, TN and Department of Chemistry, University of Kaiserslautern, FRG.

BDH is a lipid-requiring enzyme of the mitochondrial (M) inner membrane. ApoBDH [devoid of phospholipid (PL)] is inactive. ApoBDH reconstituted with PL vesicles requires its allosteric activator phosphatidylcholine (PC) to be fully active; BDH with phosphatidylethanolamine/diphosphatidylglycerol vesicles (BDH-PE/DPG) exhibits partial function and weak nucleotide and substrate binding (see McIntyre, Cheng, Eibl & Fleischer, this volume). BDH interconverts R-3-hydroxybutyrate (BOH) and acetoacetate (AcAc) with NAD(H) as coenzyme. The BDH-MPL complex (containing PC) exhibits ordered sequential kinetics. We have now studied the effects of three substrate analogues, 2-methylmalonate (2MM), malonyl-monomethylester (MME) and malonyl-monoamide (MMA) on BDH activity. For BDH-MPL (PC-activated complex), 2MM and MME are competitive inhibitors vs BOH and are either uncompetitive (2MM) or non-competitive (MME) vs AcAc; MMA is uncompetitive vs BOH and competitive vs AcAc. By contrast, with non-activated BDH-PE/DPG, 2MM and MME enhance the forward kinetics with BOH as substrate (apparent K_m for BOH is reduced ~3-4 fold) but do not affect the reverse; MMA enhances the reverse reaction only. These results suggest that, in the absence of activating PC, the substrate analogs are partially able to shift the allosteric equilibrium, thus enhancing the activity of BDH-PE/DPG. [Supported in part by NIH DK 14632]

W-Pos182

CUSTOMIZATION OF BIOCHEMICAL MICELLE SIZE. John F. Hunt, Mikio Kataoka, Tetsuro Fujisawa, and Donald M. Engelman, Yale University.

Biochemical and structural characterization of integral membrane proteins requires solubilization in detergent, a process which produces protein/detergent complexes referred to as mixed micelles. The bound detergent molecules make a critical contribution to the size and the physical properties of mixed micelles. We have developed techniques to characterize and manipulate the size of these complexes. In particular, the effect of the small amphiphile 1,2,3-heptanetriol on the size of non-ionic detergent micelles has been studied using small angle x-ray scattering and HPLC gel filtration chromatography. Both techniques show approximately a 50% reduction in the volume of β-octylglucoside (β-og) micelles in the presence of moderate concentrations of the small amphiphile (3 to 5% weight volume). The physical chemistry of the effect has been characterized in some detail. A simple HPLC gel filtration assay has been developed which shows that the effect is mediated by a low affinity binding of the small amphiphile to the micelle; this technique produces a straightforward estimate of the stochiometry of the binding (approximately a 1:3 molar ratio of 1,2,3-heptanetriol to β-og monomer at 5% heptanetriol). Finally, HPLC gel filtration has been used to show that heptanetriol also reduces the size of certain protein/detergent mixed micelles. This effect may explain the efficacy of heptantriol in promoting the crystallization of integral membrane protein complexes. Furthermore, the effect may prove to be generally useful in the fields of membrane protein biochemistry and biophysics.

W-Pos183

PROCESSING AND STRUCTURE OF MEMBRANE BOUND COAT PROTEIN SPECIES USING NMR. P. Schrader, K. Shon, J. Fowler, Y. Kim, J. Tomich, J. Richards, and S. Opella. *Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 USA; Division of Medical Genetics, Childrens Hospital of Los Angeles, Los Angeles, CA 90027 USA; Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125 USA.*

The structure and dynamics of the procoat and coat proteins from the filamentous bacteriophages Pf1 and M13 are being described with solution and solid-state NMR techniques. Protein samples prepared by solid-phase peptide synthesis and isolation from bacterial growths allow for specific, selective and uniform labeling of the protein species. Comparisons between the coat protein and procoat protein characterize the leader sequence and its influence on the entire protein. Dynamic studies using solid-state NMR and solution NMR on the procoat protein map out domains of structure and help to establish a mechanism of action for protein translocation.

W-Pos184

PHOTOLABELING OF *TORPEDO CALIFORNICA* NICOTINIC ACETYLCHOLINE RECEPTOR MEMBRANES WITH AN ARYL AZIDE DERIVATIVE OF PHOSPHATYLSERINE

Michael P. Blanton and Howard H. Wang, Department of Biology, University of California, Santa Cruz, CA 95064

A photoactivatable analog of phosphatidylserine ^{125}I 4-azido-salicylic acid phosphatidylserine (^{125}I ASA-PS) was used to label native and reconstituted *Torpedo californica* acetylcholine receptor membranes. The radioiodinated aryl azido group is attached directly to the phospholipid head group, and thus probes for regions of the polypeptide chain in the proximity of the negatively charged head group of phosphatidylserine. Previously we reported (Biochemistry, in press) that all four receptor subunits incorporated the label. The majority of the label incorporated into the α subunit was localized in fragments 11.7 and 10.1 Kdal after digestion by *Staphylococcus aureus* V8 protease. Both fragments begin at Asn-339 and are of sufficient length to contain the transmembrane region M4. ^{125}I ASA-PS incorporation into the AchR was further mapped by chemical digestion with cyanogen bromide, fragment separation by reverse phase HPLC and NH_2-terminal sequencing of radiolabeled peptides. At the time of submission of this abstract, CNBR fragments containing segments of the transmembrane regions M4 as well as M3 have been identified within radiolabeled reverse phase HPLC-purified peptides.

W-Pos185

HYDROPHOBIC PULMONARY SURFACTANT PROTEINS IN PHOSPHATIDYLCHOLINE BILAYERS. G. Simatos, K.B. Forward, M.R. Morrow and K.M.W. Keough, Departments of Biochemistry, Pediatrics and Physics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9.

Hydrophobic surfactant protein was isolated from pig lung lavage. SDS-PAGE and amino acid analysis suggest that it was primarily SP-C. Mass of this protein, previously underestimated using the fluorescamine reaction, was more reliably estimated using the Bradford method. Protein, mixed with perdeuterated dimyristoylphosphatidylcholine, enhanced the rate of adsorption at the air-water interface. NMR spectral moments indicated that protein had little effect on lipid chain order in the liquid crystal phase but reduced order in the gel phase. With protein two phase coexistence was observed over a small temperature range below the pure lipid Tm. Protein did not alter spin lattice relaxation times (T_1) much, but transverse relaxation times, T_{2e}, indicate that it influenced slow lipid motions. Protein broadened the dsc transition and decreased the Tm and the ΔH. (Supported by MRC and NSERC, Canada.)

W-Pos186

THE EFFECT OF ETHANOL ON LIPID-PROTEIN INTERACTIONS IN RECONSTITUTED MEMBRANES CONTAINING THE ACETYLCHOLINE RECEPTOR

V.C. Abadji*, D.M. Fraser#, K.W. Miller* and A. Watts#. Intro. by Martin C. Carey
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Ethanol acts on post-synaptic ion channels including the nicotinic acetylcholine receptor (nAcChoR), possibly by perturbing the lipid-protein interface. To study the effects of ethanol specifically on the nAcChoR-lipid interface, the protein was reconstituted in dioleoyl-phosphatidylcholine at various lipid to protein ratios. Spin-labeled phosphatidylcholine (14-PCSL) was incorporated into these membranes and EPR spectra were recorded over a range of ethanol concentrations, including that causing general anesthesia. Spectra exhibited two distinct components corresponding to the bulk lipid bilayer and to the motionally restricted lipid at the nAcChoR interface. Spectral subtraction and simulation were employed to quantitate the two components and the exchange of lipid between the two motionally distinct environments. As the ethanol concentration increased the fraction of lipid at the nAcChoR interface decreased with concomitant small effects on the exchange rate between the interfacial and bulk lipid. Supported by an N.I.A.A.A. grant #07040 to K.W.M. and by a S.E.R.C. grant GR/D 69846 to A.Watts.

W-Pos187

The Thermodynamic Parameters of the Binding of Retinol to Binding-Proteins and to Membranes. Noa Noy, Dept. of Medicine, Cornell University Medical College, New York, NY 10021.

Retinol, being a hydrophobic compound, distributes in vivo mainly between binding proteins and cellular membranes. To better clarify the nature of the interactions of retinol with these phases, the temperature-dependence profiles of the binding of retinol to retinol binding protein (RBP), serum albumin, unilamellar vesicles of dioleoyl phosphatidylcholine and hepatocyte plasma membranes were studied. Binding of retinol to RBP was characterized by a large increase in entropy ($T\Delta S^\circ$ = +10.32 Kcal/mol) and no change in enthalpy. Binding to albumin was driven by enthalpy (ΔH° = -8.34 Kcal/mol) and accompanied by a decrease in entropy ($T\Delta S^\circ$ = -2.88 Kcal/mol). Partitioning of retinol into unilamellar vesicles and into plasma membranes was stabilized both by enthalpic (ΔH° was -3.3 and -5.5 Kcal/mol, respectively) and by entropic ($T\Delta S^\circ$ was +4.44 and +2.91 Kcal/mol, respectively) components. The implications of these findings will be discussed. Supported by The American Cancer Society (# BC-551).

W-Pos188

LIPID COMPOSITION MODULATES THE CALCIUM SENSITIVITY OF THE BINDING OF ENDONEXIN TO MEMBRANES. Matthew Junker and Carl E. Creutz, Biophysics Program, Univ. of Virginia, Charlottesville, VA. 22908

Endonexin is a member of the annexin family of calcium dependent membrane binding proteins, all of which can bind to pure lipid membranes. Endonexin exhibits varying calcium sensitivities for binding membranes of different lipid composition. The phosphate group of phospholipids and the presence of any exposed negative lipid charge stabilize the endonexin-membrane interaction; large phospholipid head groups appear to weaken the interaction. For multicomponent vesicles containing one lipid type not bound by endonexin, the binding capacity and calcium sensitivity is determined by the mol% of lipid that is bound by endonexin. There is an estimated ratio of 8-10 phospho-glycerol moieties of the bound lipid per endonexin protein at saturation. Mixtures of different types of bound lipids in the same vesicle act synergistically to reduce the calcium requirement for endonexin-membrane binding. The data are consistent with the endonexin molecule containing multiple, cooperatively interacting specific sites to bind membrane lipids.

W-Pos189

LOCATING OLEIC ACID BINDING SITES ON SERUM ALBUMIN BY ^{13}C NMR. James A. Hamilton, Shastri P. Bhamidipati, Seiichi Era and Roberta G. Reed, Dept. of Biophysics, Boston Univ. School of Medicine, 80 E. Concord St., Boston MA and Mary Imogene Bassett Hospital, Cooperstown NY

We have investigated the binding of ^{13}C carboxyl-enriched oleic acid (OA) to two large complementary fragments of bovine serum albumin (BSA), PB (1-306) and PA (307-583), by ^{13}C NMR spectroscopy. PB showed a single peak at 180.7 ppm for the first mole of OA. PA showed two peaks of nearly equal intensity at 182.0 and 182.3 ppm for the first two moles of OA. These three peaks are the major peaks seen for native BSA with 2-3 moles OA [Parks et al., (1983) J. Biol. Chem. 258, 9282]. When the two fragments were mixed in an equimolar ratio with a total of 5 moles of OA to form a non-covalent complex, the resultant spectrum was strikingly similar to that of native BSA with 5 OA. Our results suggest the following: BSA contains one strong site in the amino domain and two strong sites in the carboxy domain. The structure of these binding sites appears to be unaffected by cleavage in the middle domain. Two medium affinity sites are present in the middle domain and/or other regions which are conformationally altered by cleavage into PA and PB.

W-Pos190

SPECTRIN STRUCTURE AND ITS INTERACTION WITH LIPID BILAYERS. R.C.MacDonald, N.K.Subbarao, K.F.Ahrens & R.I.MacDonald., Dept. Biochem., Mol. Biol. & Cell Biol. Northwestern Univ., Evanson, IL 60208

We have examined structural features of spectrin which may provide clues about its interaction with acidic phospholipids. The repeated domains of spectrin contain conserved tryptophan residues that are useful as intrinsic fluorescent probes. Most tryptophans are in hydrophobic environments, as indicated by their emission maxima, although a portion exhibit a red-shifted shoulder. Tryptophans are not quenched by the polar species, iodide ion and N-methyl nicotinamide but they are effectively quenched by hydrophobic molecules, bromostearate, acrylamide, and toluidino-naphthalene sulfonic acid. According to the emission spectrum and increased quenching by iodide and methylnicotinamide, 4 M urea partially exposes at least some tryptophans. Spectrin binds to phosphatidylserine with an affinity constant of about 10^6/M, but the interaction seems not to involve hydrophobic penetration of the bilayer, as revealed by lack of quenching by brominated phosphatidylcholine embedded in the phosphatidylserine bilayer. This is contrary to conclusions of others who used bromostearate, which we find readily exchanges between hydrophobic sites. Not all domains of spectrin bind equally well to acidic lipid surfaces. Analysis of the binding of peptides generated by subtilisin digestion revealed a 30 kD fragment with a higher affinity than any others. This fragment has been isolated for sequencing. Supported by NIH DK36634.

W-Pos191

INFLUENCE OF SIGNAL PEPTIDES ON LIPID MORPHOLOGY. Jeffrey D. Jones, C. James McKnight and Lila M. Gierasch; Departments of Pharmacology and Biochemistry, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Tx 75235-9041.

Signal peptides have recently been shown to induce bilayer to non-bilayer phase transitions in lipid dispersions under certain conditions (Batenburg et al., *Biochemistry* 27, 5678, 1988). We have employed ^{31}P NMR spectroscopy to examine the effect of a family of peptides derived from the signal sequence of the *E. coli* LamB protein on lipid morphology. These peptides differ in length and hydrophobicity, and have been shown to differ in α-helix content when bound to lipid vesicles. Also, different modes of peptide-vesicle interaction (surface binding, insertion) have been observed. Thus, the influence of both secondary structure and the nature of vesicle interaction on the capacity of these peptides to promote non-bilayer structures have been examined. Preliminary results indicate that bilayers comprised of POPE-POPG (65-35 mole%) are stable upon addition of LamB wild type signal peptide, which is insertion active and adopts a relatively high content of α-helix. Comparative studies of LamB mutants which have decreased proportions of α-helix and lower insertion capacities will also be discussed. Supported by NIH grant (GM-34962) and NSF grant (DCB-8947252).

W-Pos192

ORIENTATION OF A MODEL ION CHANNEL PEPTIDE IN LIPID VESICLES. Laura A. Chung, William F. DeGrado and James D. Lear, E.I. DuPont de Nemours & Co., Central Research & Development Dept., Experimental Station, Wilmington, DE 19880-0328

A model peptide, H_2N-(Leu-Ser-Ser-Leu-Leu-Ser-Leu)$_3$-$CONH_2$, was previously shown to form voltage-dependent ion channels (J.D. Lear, et al.(1988) Science 240,1177). We have synthesized seven variants in which a residue in the central heptad has been replaced by a tryptophan. Interactions of these peptides with large and small phospholipid vesicles were studied using fluorescence. Our results indicate that when the peptide:lipid ratio is 1:100 and no transmembrane voltage is present, the peptide resides in the membrane as an alpha helix. Trp residues which replace Leu in the sequence are shown to be in a hydrophobic environment whereas Ser replacements are in a hydrophilic environment. Quenching results using lipid-linked quenchers were consistent with a surface rather than transbilayer orientation of the peptide under conditions where no channel activity is expected. Thus, the voltage-dependence of the channel opening in planar bilayers may involve a voltage-induced change in peptide orientation from a surface to a transbilayer configuration.

W-Pos193

THE INTERACTIONS BETWEEN CYTOCHROME C AND MODEL MITOCHONDRIAL LIPID VESICLES.

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Isothermal titration calorimetry and quasi-elastic light scattering have been used to determine the energetics of association to model mitochondrial lipid vesicles (PC and Cardiolipin) for both the ferric and ferrous forms of cytochrome c. In addition, high sensitivity differential scanning calorimetry (DSC) has been used to analyze the effect of cytochrome c on the gel to liquid crystalline transition in model mitochondrial lipid systems.

Experiments performed by DSC show that cytochrome c undergoes a reversible, two-state unfolding transition. At increasing membrane concentrations, however, the single peak observed in the first scan gives rise to a double peak in subsequent scans. This behavior is consistent with the idea that the membrane associated unfolded cytochrome c refolds into a state distinct from that of the free protein. (Supported by NIH grants GM-37911 and RR-04328).

W-Pos194

DISTRIBUTION OF TRIPEPTIDES BETWEEN OCTANOL AND WATER. A. Kim & F. C. Szoka, Jr., School of Pharmacy, UCal., San Francisco, CA.

Prediction of protein structure and protein-membrane interactions from amino acid sequence have been based upon both theoretical and experimental measurements. Experimental data for certain hydrophilic amino acids is not available over a range of relevant pH values. We have measured the distribution of tripeptides of the sequence n-^{14}C-acetyl-A-X-A-tbutylamide between octanol and water as a function of temperature and pH. The central amino acid was either A, G, P, F, W, H, D, or E.

residue	ΔH (Kcal/mole)	ΔS (cal/mole°K)	ΔG (Kcal/mole)
G	6.2	22.3	-0.46
A	7.2	26.4	-0.57
F	6.0	29.2	-2.65
W	3.4	21.4	-2.99
P	8.3	30.4	-0.75
H pH3	2.2	2.1	1.55
H pH9	4.8	18.2	-0.66
D pH2	5.0	13.0	-0.33
D pH9	3.4	0.7	3.26
E pH2	5.1	18.4	-0.42
E pH9	2.9	0.46	2.77

The observed ΔG values agree well with values calculated by the fragment method as described by Abraham and Leo(Proteins:Struc.,Func.&Gen. 2:130-152. 1987).

W-Pos195

FACTORS AFFECTING THE MEMBRANE TRANSLOCATION OF DIPHTHERIA TOXIN. Zohreh Toossi Farahbakhsh and Bernadine J. Wisnieski. Department of Microbiology and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

Although several models have been proposed for toxin entry into the cytoplasm, the precise mechanism by which fragment A gains access to elongation factor 2 and the identity of the peptide regions that participate in membrane traversal are not known. This study was conducted to explore the functional role of low pH and to determine whether changes in toxin structure continue to occur after acid-dependent binding/insertion is achieved, i.e., changes specifically induced by contact with membrane lipids.

Comparisons between membrane-bound toxin and acid-pulsed soluble toxin enabled us to identify regions of the toxin that are translocated across the bilayer, and factors (i.e., temperature, membrane physical state, toxin cleavage and reduction) that regulate the migration of specific segments of the toxin molecule. Acid-triggered structural changes in both A and B domains of intact toxin lead to their membrane insertion, with subsequent structural changes occurring upon lipid contact and toxin cleavage. Cleavage alters protein migration.

Supported by USPHS GM22240 and UCLA Academic Senate.

W-Pos196

A MOLECULAR MECHANISM FOR THE BINDING OF PROTEIN KINASE C TO MEMBRANES. M. Mosior, J. Kim, L. Chung, H. Wu, & S. McLaughlin. Dept. of Physiology & Biophysics, HSC, SUNY, Stony Brook NY 11794.

Work from many different laboratories suggests that the translocation of PKC from the cytoplasm to the plasma membrane may be due to the interaction of one PKC with several negatively charged PS lipids. 5 of the first 11 amino acids in the highly conserved C_1 region of PKC are positive: the sequence is RFARKGALRQK. We investigated the binding of this and other small synthetic peptides to phospholipids by making zeta, surface potential and fluorescent probe measurements. The binding could be described by the Gouy-Chapman-Stern theory. If we assume the peptide:PS stoichiometry is 1:1, the data for di, tri, tetra and pentalysine and the PKC peptide can be described with intrinsic association constants of 10, 10^2, 10^3, 10^4 and 10^4 M^{-1}. However, diluting the PS with PC, to which the peptides do not bind, decreases the strength of the binding, which we can describe theoretically by assuming the peptides bind to more than one negative lipid. Our results support the NMR studies of Roux et al. (1988), which suggest pentalysine binds outside the envelope of the headgroups. The binding of the PKC peptide is sufficiently strong to be an important factor in the distribution of PKC between the cytoplasm and membrane.

W-Pos197

ELECTROSTATIC ASSOCIATION OF PHOSPHOLIPASE C WITH NEGATIVELY CHARGED MEMBRANES. Arnold A. Peterson, Mario J. Rebecchi, and Stuart McLaughlin. Dept. of Physiology & Biophysics, HSC, SUNY, Stony Brook, NY 11794-8661

PLC, phosphoinositide-specific phospholipase C, can be isolated from both the cytoplasm and plasma membrane fractions of cells. We hypothesize that a highly conserved, positively charged (5+) region of PLC (QLRGKILLKGKKL) binds to negatively charged lipids (e.g. PS) on the inner monolayer of the plasma membrane. We estimated the binding of a synthetic peptide with this sequence to PC:PS vesicles by making electrophoretic mobility measurements. The binding could be described by the Gouy-Chapman-Stern theory with a peptide-PS intrinsic association constant of order 10^3 M^{-1}. We estimated the binding of PLC (85 kD) to PC:PS vesicles by measuring the enzymatic activity of PLC toward vesicles containing PIP_2 in the presence of competing PC:PS vesicles. PLC and the peptide bound with similar affinities to 2:1 and 5:1 PC:PS vesicles. Although this electrostatic binding is strong enough to affect the distribution of PLC between the cytoplasm and the plasma membrane, other factors must be important in the cell. Our studies reveal that additional (hydrophobic?) binding occurs with phospholipid vesicles on a time scale of minutes.

W-Pos198

PRODUCT FATTY ACID INHIBITS THE ADSORPTION OF CARBOXYLESTER LIPASE TO PHOSPHATIDYLCHOLINE-CONTAINING SURFACES. J.M. Muderhwa and H.L. Brockman, Hormel Institute, Univ. of MN, Austin, MN 55912.

For water-soluble carboxylester lipase (CEL) to function, it must first bind to the lipid-water interface. For binary mixed films of 1-palmitoyl-2-oleoyl-*sn*-2-glycerophosphocholine (POPC) and substrate (S), there is a mol fraction (X_s) range for each S over which S hydrolysis by native CEL is very low, even at high [CEL]. Within this range and at high [CEL], the surface concentration of native CEL (Γ_{CEL}) was 0.18 pmol/cm^2 at X_s=0 and increased with X_s for each S to $\leq$0.6 pmol/cm^2. Because Γ_{CEL} was measurable with POPC alone, the data were analyzed by a free+excluded area model. For all S, the excluded area was 43-45 Å/POPC. In contrast, in films with product fatty acid (P) in place of S, Γ_{CEL} was 0.18 pmol/cm^2 at X_p=0 but remained unchanged up to X_p~0.5, above which it increased with X_p. Because area generated by S hydrolysis to P up to X_p~0.5 is unavailable for CEL adsorption, S hydrolysis by CEL or other lipases would inhibit additional CEL binding. Supported by NIH Grant HL17371.

W-Pos199

INTERACTION OF CYCLOSPORIN A WITH DIPALMITOYLPHOSPHATIDYLCHOLINE AT THE AIR/WATER INTERFACE. Timothy S. Wiedmann, Kimberly R. Jordan, University of Minnesota, Department of Pharmaceutics, Minneapolis, MN
(Introduced by Eugene Grim)

Surface pressure-surface area isotherms with the whole range of mole fractions of cyclosporin A (CyA) in DPPC have been determined. Pure CyA exhibits an isotherm which rises gradually and undergoes a transition at about 15 dyn/cm. This transition appears to result from a combination of molecular rearrangement and loss to the subphase deduced from recycling the monolayer and from the value of the equilibrium spreading pressure which was 30 dyn/cm. CyA is shown to form nonideal films at low surface pressures, and the limiting area is about 140 Å^2/molecule with considerable attractive interactions. The molecular area as a function of mole fraction of CyA in DPPC indicates that minor deviations from ideal mixing occur at the low mole fractions. The results suggest that CyA induces disorder in the acyl chains of DPPC thereby giving rise to nonideality. Work conducted at the Center for Interfacial Engineering, U of M.

W-Pos200

CONFORMATION AND FUNCTION OF LIPID-BOUND CYTOCHROME C IS CONTROLLED BY PHYSICAL STATE AND COMPOSITION OF THE MEMBRANE.
T.R.Heimburg,D.Marsh and P.Hildebrandt,Max-Planck-Institut f.biophys.Chemie,D-34 Goettingen,F.R.G.

The conformational states cI and cII with redox potentials of 0.0 and -0.4V,respectively, of cytochrome C bound to vesicles of dimyristoyl phosphatidylglycerol(DMPG) and dioleoyl phosphatidylglycerol(DOPG)have been investigated by resonance Raman spectroscopy. The thermotropic phase transition of DMPG at 23°C changes the ratio cII/cI of the conformatinal populations from 4.2 in the gel phase to 6.0 in the liquid crystalline phase.Cytochrome C bound to DOPG(T_m=-20°C) showed no change at 23°C(cII/cI=2.8).Thus the main transition is responsible for the change in cII/cI.In mixtures of dioleoylglycerol(DOG) with DOPG the ratio cII/cI decreased continuously from 2.8 to 1.1 for 0 to 30mol% DOG.The change in cII/cI is not directly correlated to variations in the area and charge density of the membrane surface.^{31}P-NMR results strongly suggest that these changes are due to local structural changes at the protein binding site.Similar observations have been made in mixtures of dioleoyl phosphatidylcholine with oleic acid and DOPG.Therefore the ratio cII/cI can be influenced significantly by the phase state and the chemical composition of the membrane. This effect is proposed to be of general importance in controlling the function of other proteins,such as protein kinase C and phospholipase A2.

W-Pos201

THE STRUCTURE OF THE HYDROPHOBIC DOMAIN OF CYTOCHROME B5 BY FOURIER TRANSFORM INFRARED SPECTROSCOPY. Peter W. Holloway and Catherine Buchheit, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

Cytochrome b_5 is bound to membranes by a hydrophobic C-terminal domain of approximately 40 amino acids (NPP, nonpolar peptide). Our earlier FTIR studies of cytochrome b_5 in aqueous solution indicated the NPP is predominantly alpha helical. For greater sensitivity we are using the isolated NPP in the present studies. FTIR spectra of the NPP were obtained in D_2O, and after the addition of POPC, DMPC or DOC. The spectra were subjected to self deconvolution and curve fitting to obtain the contributions of the individual amide I peaks to the broad amide I contour of the NPP. The percentages of alpha helix in the four situations were: 45, 57, 56 and 60. In buffer alone, or in lipids, the major amide I band was at 1656 cm^{-1} (unexchanged alpha helix). In contrast, the major peak in DOC was at 1650 cm^{-1}, indicative of exchanged alpha helix. The extent of NH to ND exchange was confirmed by changes in the intensity of the amide II band. These results indicate that in the membrane bound form of NPP the majority of the peptide backbone is protected from the bulk D_2O. Supported by NIH GM 23858.

W-Pos202

LOW PH INDUCED CHANGES IN PSEUDOMONAS EXOTOXIN CONFORMATION AND HYDROPHOBICITY INVOLVE A TWO STEP PROCESS
Xin Jiang and Erwin London, Dept. of Biochemsitry and Cell Biology, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794-5215

Previous investigators have shown that exotoxin A undergoes a conformational switch to a hydrophobic state at low pH. This change is believed to play a role in exotoxin cell entry by facilitating its penetration of the membranes of acidic organelles. We have examined the low pH changes in exotoxin using fluoresence, calorimetric and proteolytic techniques. A two step process occurs as pH is decreased, with the first conformational transition at pH 5-5.5 and the second at pH 4. Exotoxin remains in a largely folded state after the first transition. In contrast, the second transition involves denaturation-like changes. In addition, exotoxin A becomes hydrophobic after the second transition, as shown by insertion into detergent micelles and model membrane vesicles. The pH at which exotoxin inserts into vesicles depends on lipid composition, and insertion can occur at pH 5 in the presence of vesicles containing 20% negatively charged lipids. Supported by NIH grant GM 31986.

W-Pos203

APOCYTOCHROME C INTERACTION WITH PHOSPHOLIPID MEMBRANES STUDIED BY FT-IR SPECTROSCOPY. *W.K. Surewicz, A.Muga & H.H. Mantsch*, Division of Chemistry, National Research Council, Ottawa, K1A 0R6, CANADA

Apocytochrome c, the heme-free precursor of cytochrome c, has been extensively used as a model to study the molecular aspects of posttranslational translocation of proteins across membranes. In this report, we have used FT-IR spectroscopy to gain further insight into the mechanism of the lipid-apocytochrome c interaction. Association of apocytochrome c with a model membrane composed of DMPG and acyl-chain perdeuterated DMPC-d_{54} results in an increased conformational order of the lipid acyl chains,particularly at temperatures above the phase transition. The lipid phase transition is significantly broadened, although it remains detectable by the FT-IR method even in the presence of saturable amounts of the protein. The properties of both lipid components of the membrane are perturbed in a very similar fashion, indicating that there is no phase separation between DMPG and DMPC-d_{54}. Binding to phospholipids results in a dramatic change in the secondary structure of apocytochrome c. In the presence of a large excess of phospholipid vesicles (or micelles of SDS), there is a transition from an unordered structure to α-helix. However, at lower lipid-to-protein ratios apocytochrome c adopts an extended β-sheet structure, suggestive of protein aggregation on the membrane surface.

W-Pos204

PHOSPHATIDYLCHOLINE TRANSFER PROTEIN ENHANCES MICROSOMAL SYNTHESIS OF PHOSPHATIDYLCHOLINE

Zafar U. Khan & George M. Helmkamp, Jr.

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66103

Cholinephosphotransferase (CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase, EC 2.7.8.2), is the final enzymatic step in the *de novo* pathway for synthesis of phosphatidylcholine (PtdCho). PtdCho transfer protein, a cytosolic protein, is capable of transporting PtdCho between isolated cellular membranes or vesicles. Rat liver PtdCho transfer protein stimulates liver or brain microsomal cholinephosphotransferase activity 4-8 fold. Gradient-purified rough and smooth endoplasmic reticulum exhibit the same degree of stimulation. We observed PtdCho synthesis is also enhanced by bovine liver PtdCho transfer protein. Within a series of diacylglycerol substrates having different acyl moieties, the extent of stimulated PtdCho synthesis correlates with the intermembrane transport activity of PtdCho transfer protein toward PtdCho molecules of different fatty acyl composition. The stimulatory property of PtdCho transfer protein is not diminished after partial proteolytic digestion. These data describe a novel property of PtdCho transfer protein which may be of physiological significance in the regulation of mammalian PtdCho synthesis.

(This work was supported by NIH grant GM24035)

W-Pos205

ELUCIDATION OF SLOW MOTIONS IN GLYCOGLYCEROLIPID BILAYERS BY TWO-DIMENSIONAL SOLID-STATE DEUTERON NMR.

Michèle Auger and Harold C. Jarrell, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada, K1A 0R6.

Two-dimensional solid-state deuteron NMR spectroscopy has been used to demonstrate the presence of a slow whole molecule motion in the gel phase of hydrated multibilayers of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL). This technique provides an observation window in a frequency range ($< 1 \times 10^3$ s^{-1}) not accessible from lineshape and spin-lattice relaxation study. The results clearly reveal the presence of a slow motion in the gel phase of the glycolipid β-DTGL at 35°C. The correlation time for the motion was determined to be of the order of milliseconds. Comparison of the experimental and simulated two-dimensional ridge patterns suggest that a large angle jump about the long molecular axis can best account for the 2D exchange spectra of β-DTGL in the gel phase in comparison to small step Brownian diffusion. This conclusion is in agreement with previous studies suggesting that large angle jumps may be favoured in highly ordered systems.

W-Pos206

ELUCIDATION OF MOTIONAL MODES IN GLYCOLIPID AND PHOSPHOLIPID BILAYERS: A ^2H NMR LINESHAPE AND RELAXATION STUDY.

Michèle Auger, Marie-Rose Van Calsteren, Ian C.P. Smith and Harold C. Jarrell, Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada, K1A 0R6.

^2H NMR lineshape and spin-lattice relaxation analysis has been used to investigate the dynamics of several phospholipid and glycolipid bilayers. The gel phase spectra of these lipids labelled at the C3 position of the glycerol backbone are broad (≈ 90 kHz) and characteristic of fast-limit axially asymmetric motion. Moreover, anisotropic spin-lattice relaxation was observed in all systems. The lineshape and relaxation features in the gel phase were best simulated using a fast-limit three-site jump model, with relative site populations of 0.46, 0.34 and 0.20. This motion was associated with an internal jump about the C2-C3 bond of the glycerol backbone. A second motion, namely rotation about the long axis of the molecule as a whole, was needed to account for the observed temperature dependence of the quadrupolar echo amplitude and the spectral lineshape below to above the gel to liquid-crystalline phase transition temperature.

W-Pos207

COMPUTER MODELLING OF LIPID MEMBRANE PHASE TRANSITIONS

D.P. Fraser, O.G. Mouritsen‡ and M.J. Zuckermann. *McGill University, Montreal,* ‡ *The Technical University of Denmark, Lyngby, Denmark.*

In order to make a realistic numerical simulation of the translational and conformational degrees of freedom of lipid acyl chains, we have developed a new model of biological membranes in which the constituent molecules are mapped onto a plane parallel to the membrane surface. In the two-dimensional analogue of the membrane, the particles represent the cross-sections of lipid acyl chains. The Voronoi tesselation of this system is incorporated into constant-NPT Monte-Carlo simulations and provides easy access to nearest and next-nearest neighbours. In addition, Voronoi statistics provide a method for examining the liquid/solid structure of the systems. The tesselation is dynamically maintained during the simulations.

Studies have been made on binary mixtures of hard-core discs in which the concentrations of the species are allowed to vary according to pre-set degeneracies. These mixtures provide a minimal model for the lipid systems in that they give a transition from a liquid phase in which the large discs predominate to a crystalline state in which the small discs predominate. This mimicks the phase behaviour of pure lipid systems.

A complete single-chain density of states is used to give an improved description of the main phase transition and extensions to include integral protein and cholesterol molecules will be discussed.

W-Pos208

PHASE TRANSITIONS IN DRY PHOSPHATIDYL ETHANOLAMINE

C. Aurell W, L.M. Crowe, J.H.Crowe, Univ of Calif, Davis

The thermotropic inverted hexagonal phase transition, TH_{II}, in dry pure PE is decreased to -5 °C compared to the fully hydrated transition at about 30 °C. In presence of carbohydrates this dry transition is increased to a temperature well above the hydrated transition. In presence of solutes like proline and urea the Tm in dry PE is lowered and the inverted hexagonal phase transition TH_{II} is elevated. Evidence from differential scanning calorimetry, freeze fracture and fourier transform infrared spectroscopy are shown. In dry mixtures of eggPE/DPPC and eggPE/eggPC thermotropic transitions measured with DSC, suggest that presence of carbohydrates prevent phase separation. Lipid mixtures dried in presence of ionic solutes show separation of temperature transitions.

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NSG RA/62

W-Pos209

HAND-DRIVEN, SMALL-VOLUME EXTRUSION APPARATUS FOR LUVET PREPARATION. R.C. MacDonald, R.I. MacDonald, B. Menco, N. Subbarao, K. Takeshita and L.-r. Hu. Intro. by I.M Klotz. Dept. Biochem., Cell Biol. and Mol. Biol., Northwestern University, Evanston, IL 60201.

We have designed and constructed a filter-holder which is hand-driven, easily made and will support high pressure extrusion of tenths of milliliter volumes of multilamellar vesicles. The MLVs were prepared by hydrating egg phosphatidylcholine at 6-30 mM and freeze-thawing them ten times. By slam freeze-fracture electron microscopy, the extruded vesicles were unilamellar with average diameters of 69 $\pm$ 19 and 80 $\pm$ 24 nm when extruded through 100 nm pore filters and average diameters of 48 $\pm$ 12 and 54 $\pm$ 20 nm when extruded through 30 nm pore filters. These dimensions agreed with those obtained by treating the LUVETs with negative stain. To see whether the extrusion per se causes the bilayers to rupture, we added the fluorescent dye, calcein, at different stages of the extrusion procedure. The net gains in trapped volume were 1.1 μl/μmole lipid due to freeze-thawing, 1.45 μl/μmole lipid due to the first extrusion and 0.15 μl/μmole lipid due to subsequent extrusions. Thus, the vesicles become permeable to and trap calcein only during the first extrusion and not during subsequent extrusions. LUVETs prepared with our mini-extruder appear to be equivalent to and more easily made than those prepared with a more expensive, commercially available device. Supported by NIH Grant GM38244.

W-Pos210

FLUORESCENCE-BASED METHOD TO MONITOR LIPID LATERAL DISTRIBUTIONS IN LIPID BILAYERS John R. Silvius
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The fluorescence of carbazole-labeled phospholipids is quenched by lipids with dibrominated acyl chains, with an efficiency that is highly sensitive to the mutual lateral distributions of the carbazole-labeled and the brominated lipid species, in bilayers combining brominated and nonbrominated lipids. We have used this effect to monitor thermotropic and ionotropic lateral phase separations in lipid vesicles labeled with 1-palmitoyl-2-(11-carbazoleundecanoyl)-phosphatidylcholine and composed of various mixtures of 1-palmitoyl-2-(11,12-dibromooctadecanoyl)-phospholipids and nonbrominated phospholipids. This approach has provided information on both the kinetics and the extents of calcium-induced lateral segregation of anionic lipids in vesicles combining phosphatidylcholine with various species of phosphatidylserine (PS) or phosphatidic acid (PA). We have used such information to assess the abilities of various factors to favor such phase separations, and the possible role of lipid lateral segregation in calcium-induced vesicle fusion.

W-Pos211

VOLUMETRIC PROPERTIES OF 1-STEAROYLLYSOPHOSPHATIDYLCHOLINE AQUEOUS ASSEMBLIES. Jeffrey T. Mason, The Armed Forces Institute of Pathology, Washington, D.C. 20306-6000.

Density measurements have been conducted on aqueous assemblies of 1-stearoyllysophosphatidylcholine. Upon heating, dilatometric measurements reveal a lamellar to micellar phase transition centered at 26.2°C. The volume change for this transition is estimated to be 0.075 mL/g. The volume coefficient of expansion of the lamellar phase is strongly temperature dependent and reaches a value of $0.038°C^{-1}$ immediately preceding the phase transition. This value exceeds the corresponding volume coefficient of expansion of the $P_{\beta'}$ phase of saturated diacylphosphatidylcholines by about a factor of 30. This difference is proposed to arise from a structural ordering of the hydration water associated with the lysophospholipid. The density of this hydration water is suggested to be coupled to the temperature-dependent dynamic motions of the lysophospholipid headgroup. The lipid effective specific volume in the micellar phase at 28.1°C is 0.929 mL/g. Supported by USPHS Grant GM-33040.

W-Pos212

EFFECTS OF N-PROPANOL ON DMPE BILAYERS J.A. Centeno and T.J. O'Leary, Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000

We have investigated the effects of n-propanol and increased hydrostatic pressure on dimyristoylphosphatidylethanolamine (DMPE) assemblies using differential scanning calorimetry and infrared spectroscopy. At concentrations below 0.6M, n-propanol lowered the gel to liquid-crystalline and unhydrated crystalline to liquid-crystalline phase transition temperatures by as much as 8°C and 5°C respectively. In addition, propanol increased the rate of spontaneous dehydration of the gel phase. This increase in dehydration rate was augmented by the application of hydrostatic pressures of up to 100 atm. Although low alcohol concentrations lowered the enthalpy of the gel to liquid crystalline phase transition, high concentrations markedly increased it. Infrared spectra demonstrated that the phase giving rise to this high enthalpy melting process is distinct from the crystalline and the hydrated gel phases, that it posseses increased headgroup mobility, and that it contains significant amounts of propanol in solid solution. These features are consistent with formation of an interdigitated gel phase in DMPE-alcohol-water dispersions. Lipid in this high melting phase spontaneously dehydrates at a much lower rate than does lipid in the ordinary gel phase. This work was supported by NIH grant GM40155.

W-Pos213

EFFECT OF CURVATURE INDUCED CHARGES ON THE CURVATURE ELASTICITY

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Introduced by D.L. Gilbert

Surface charges of a membrane and their electrolytic counterions play an important role for the bending energy. Recently (J. Phys. Chem. 92 (1989) 6865) we calculated within the Debye-Hueckel approximation the free energy of a membrane for three different geometries. In the case of a cylinder and a sphere a develloppment up to second order in powers of the inverse curvature has been used. Comparing the second order terms of the free energy with those of the pure mechanical curvature energy, we calculate an electrical bending modulus and the elastic modulus of Gaussian curvature.

For large Debye-lengths the curvature causes coupling of the two layers, the fieldlines penetrates the membrane and makes it less stiff, whereas the gaussian modul describing the change of topology remains unchanged. Beyond Debye-Hueckel, for large values of surface charges, the bending modulus becomes independent of the surface charge density and the Gaussian modul increases logarithmically.

Supported by the DFG through SFB312 and Wi933/2-1.

W-Pos214

PHASE BEHAVIOR OF THE *POPE-POPC* SYSTEM.

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The detailed phase behavior of the lipid system 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) : 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) : H_2O is unknown, although this system is increasingly being used as a model in studies of lipid structure and function. As a basis for studying the correlation between hydrocarbon chain order parameter profile and the spontaneous radius of curvature of the lipid layer in the inverted hexagonal (H_{II}) phase, we have determined the phase behavior of this system by x-ray diffraction in the presence and absence of alkane (dodecane) as a function of composition and temperature with excess water.

For all compositions of this system, we found a transition from the gel phase (L_β) to the liquid crystalline phase (L_α) near -8 °C, accompanied by an increase in lamellar spacing from approximately 64 A to 69 A. In the presence of 20 wt% dodecane, a transition to H_{II} phase occurred near 15 °C for mixtures ranging from 100 mol% POPE to 62 mol% POPE : 38 mol% POPC. Mixtures richer in POPC than this (up to 16:84) did not exhibit an H_{II} phase, but with increasing temperature partitioned into two apparently lamellar components with temperature dependent spacings of approximately 59 A and 65 A. Interestingly, POPC alone with 20 wt% dodecane exhibited a small amount of hexagonal phase at elevated temperature (>60 °C). The basis dimension of the hexagonal unit cell varied sharply with temperature and composition over a range of 65 A to 220 A. In the absence of dodecane, the observation of a hexagonal phase was confined to compositions very rich in POPE (>85 mol%) at elevated temperatures (>60 °C).

This work was supported in part by ONR, NIH, DOE and NSF.

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W-Pos215

QUASI-ELASTIC LIGHT SCATTERING (QELS) STUDIES OF MICELLE-TO-VESICLE TRANSITION IN AQUEOUS DETERGENT/PC MIXED MICELLAR SYSTEMS.

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Mixed micelles of phosphatidylcholine (PC) and a soluble detergent (D) form vesicles upon aqueous dilution, as a result of the dissociation of the detergent molecules from mixed micelles. In this study the micelle-to-vesicle phase transitions are studied as a function of temperature, pH, ionic strength and valency of the dilution media, PC/D molar ratio, total lipid concentration and the charge of the detergent, by QELS. The results showed that transition pattern was significantly altered with changes in temperature, pH, ionic strength, PC/D and the total lipid concentration. The transition did not occur when charged mixed micelles were diluted with water or 5% dextrose solution. However, the presence of counterions in the dilution media promoted vesicle formation. When neutral mixed micelles were diluted with water or 5% dextrose solution transition also occurred. These results suggest that both hydrophobic and electrostatic interactions between molecules are major contributors to the micelle to vesicle transition.

W-Pos216

THE EFFECT OF A CHAIN-TERMINAL CARBONYL GROUP ON GEL-STATE PACKING OF ASYMMETRIC CHAIN LENGTH PHOSPHATIDYLCHOLINES.

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Although autooxidation of 1-saturated-2-unsaturated phosphatidylcholines (PCs) are known to give PCs with an aldehyde at the terminus of a short sn-2 chain, the effects of a polar oxygen atom on hydrocarbon chain packing in bilayers are not understood. We have prepared two carbonyl-terminated PCs in which the sn-2 chain is: (1) a methyl ketone, $CH_3CO(CH_2)_7CO_2-$, and (2) a methyl ester, $CH_3OCO(CH_2)_8CO_2-$; in each compound, the sn-1 chain is stearoyl. The thermotropic phase behavior of aqueous dispersions of binary mixtures of either of the carbonyl-terminated PCs with C18:C10PC was studied by differential scanning calorimetry. In contrast to the behavior found previously for the sn-2 chain-terminated alkene C(18):C(11:1delta-10)PC[Biochemistry 28, 522 (1989)], solid-phase immiscibility was observed at low temperatures for the carbonyl-terminated PCs. The phase diagrams show nonideal mixing behavior, indicating perturbation of hydrocarbon chain packing in the gel state by the sn-2-terminal oxygen function. [Supported by HL16660]

W-Pos217

EFFECT OF DIVALENT CATIONS ON THE STRUCTURE OF DIPALMITOYLPHOSPHATIDYLCHOLINE AND PHOSPHATIDYLCHOLINE/PHOSPHATIDYLGLYCEROL BILAYERS

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The interactions of $CaCl_2$ or $MgCl_2$ with multilamellar phospholipid bilayers were studied by ^2H NMR. Two model membrane systems were used: 1) dipalmitoylphosphatidylcholine (DPPC) bilayers; 2) bilayers composed of mixture of phosphatidylcholine and phosphatidylglycerol at a molar ratio of 5:1. Addition of 0.25 M $CaCl_2$ to DPPC bilayers resulted in significant uniform increase of the order parameters of the lipid side chains; the effect of 0.25 M $MgCl_2$ was insignificant. Both phosphatidylcholine and phosphatidylglycerol components of the mixed bilayers were affected by the presence of 0.25 M $CaCl_2$ and, to a much smaller degree, by $MgCl_2$. Unexpectedly, the addition of Ca^{2+} induced significantly larger increase of the order parameters of the phosphatidylcholine component. The results are consistent with the long-range effects of Ca^{2+} binding on the packing of the lipid membranes.

W-Pos218

CHARACTERIZATION OF THE TRANSVERSE RELAXATION RATES IN LIPID BILAYERS.

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The deuterium NMR transverse relaxation rates of a deuterated phospholipid bilayer reflect slow motions in the bilayer membrane A study of dimyristoyllecithin (DML) specifically deuterated at several positions of the hydrocarbon chains indicates that these motions are cooperative and are confined to the hydrocarbon chains of the lipid bilayer. However, lipid headgroup interactions do play an important role in modulating the properties of the cooperative fluctuations of the hydrocarbon chains. In mixed lipid-DML systems, lipids with small headgroups do not perturb the cooperativity of director fluctuations in the DML bilayer; neither do lipids with very different hydrophobic moieties. Lipids with large headgroups, however, interfere greatly with these cooperative motions. Incorporation of a peptide such as gramidicin A' in the bilayer also disrupts the cooperative director fluctuations characteristic of the pure multilamellar lipid dispersions.

W-Pos219

THE ENERGY DIFFERENCE BETWEEN LIPID AGGREGATE STRUCTURES.

Irina Vayl. Brown University. Providence. RI.

The change of energy in the process of structure evolution of the lipid membrane in water solution is the topic of this work. The planar lipid membrane is transformed under some conditions to micelle, vesicle or other lipid aggregates. Here we try to estimate the change of energy due to transformation of the planar lipid membrane to other lipid aggregates of the correct geometry (spherical, cylindrical,etc.).

The model is based on the molecular mechanic and thermodynamic approaches. The electrostatic and Van-der-Waals energy interactions between two oriented lipid molecules were calculated using CHARMM. The potential curve for the two-particle interaction was applied to calculate the surface energy. For that purpose we will classify the molecular interactions by identical types and introduce the graph representation for them. The minimum energy corresponds to the state at absolute zero (the third law of thermodynamics). So, the density of the surface energy can be accurately determined for different types of lipid aggregates.

Now we can evaluate the coefficient of surface tension and determine the dielectric constant in which those aggregates would arise.

All calculations are done for DPPC molecules.

W-Pos220

PHOSPHATIDYLETHANOLAMINE INDUCED DESTABILIZATION OF PHOSPHATIDYLCHOLINE BILAYERS:

S.W. HUI, K.H. CHENG, T.V. ISAC, W. WHITFORD AND A. SEN, Membrane Biophysics Laboratory, Roswell Park Memorial Institute, Buffalo, N.Y. 14263.

Phosphatidylethanolamine (PE) due to its tendency to form inverted hexagonal (H_{II}) phase can destabilize phosphatidylcholine bilayers and at sufficiently high concentrations can induce the formation of H_{II} and other non-bilayer structure in PE/PC mixtures. The destabilization of POPC bilayers by increasing concentrations of DLPE was studied by measuring their susceptibility to phospholipase A_2 and the rate of lipid exchange by phospholipid exchange proteins. Both multilamellar and large unilamellar vesicles (MLV and LUV respectively) were used for these measurements. In MLV's both phospholipase A_2 and phospholipid exchange proteins showed increased activity with increasing DLPE concentration which peaked at ~82.5-85% DLPE. In LUV preparations also there was similar effect of DLPE concentration. The maximal activity in the LUV's were however at lower DLPE concentrations (~70%) as compared to those in MLV's. These results lend support to the hypothesis that lipid packing can effect polymorphic phase behavior (Hui & Sen, PNAS 86,5825-5829, 1989).

W-Pos221

CALORIMETRIC STUDIES ON MIXED-CHAIN PHOSPHATIDYLCHOLINES WITH MW IDENTICAL TO C(17):C(17)PC. Hai-nan Lin, Zhao-qing Wang, and C. Huang, Dept. of Biochemistry, Health Sciences Center, Univ. of Virginia, Charlottesville, Va 22908.

We have semisynthesized the following mixed-chain phosphatidylcholines: C(18):C(16)PC, C(16):C(18)PC, C(19):C(15)PC, C(15):C(19)PC, C(20):C(14)PC, C(14):C(20)PC, C(21):C(13)PC, C(13):C(21)PC, C(22):C(12)PC, and C(12):C(22)PC. These ten mixed-chain PCs have a common molecular weight of 762.2 which is identical to that of C(17):C(17)PC. We have employed high-resolution differential scanning calorimetry with cooling scan capability to investigate the thermotropic phase behavior of dispersions prepared from these lipids. When the values of normalized chain length difference between the sn-1 and sn-2 acyl chains in mixed-chain PC, $\Delta C/CL$, are plotted against the T_m values for aqueous dispersions of these mixed-chain PCs, a linear function is obtained only for C(17):C(17)PC (T_m=49.0°C), C(15):C(19)PC (T_m=44.9), C(18):C(16)PC (T_m=44.4), C(14):C(20)PC (T_m=39.9), C(19):C(15)PC (T_m=38.8), C(13):C(21)PC (T_m=34.1), and C(20):C(14)PC (T_m=33.1). These results suggest that mixed-chain PCs with $\Delta C/CL$ = 0.1 - 0.4 are most likely to self-assemble into partially interdigitated bilayers at $T<T_m$; in contrast, highly asymmetric PCs ($\Delta C/CL$ = 0.44 - 0.55) are most likely to self-assemble into mixed interdigitated lamellae at $T<T_m$. In the case of C(16):C(18)PC ($\Delta C/CL$ = 0.032), the T_m value (48.8°C) can fit into the liner function, if a 2gl kink is introduced into the sn-1 chain so that the value of $\Delta C/CL \approx 0.10$, suggesting that the noninterdigitated packing may not exist in the gel-state bilayer. Supported by USPHS Grant GM-17452.

W-Pos222

STRUCTURE AND INTERACTIONS OF DIHEXADECYLPHOSPHATIDYLETHANOLAMINE (DHPE) AND DIPALMITOYLPHOSPHATIDYL-ETHANOLAMINE (DPPE). F.S. Hing, P.R. Maulik and G.G. Shipley. Biophysics Institute, Boston University School of Medicine, Boston, MA 02118.

The ether-linked phospholipid, DHPE, was studied alone as a function of hydration and in combination with the corresponding ester, DPPE, at full hydration using differential scanning calorimetry (DSC) and X-ray diffraction. By DSC from 0.0 to 10.0 wt%H_2O, DHPE displayed a single reversible transition, previously shown to be a direct $L_\beta \rightarrow H_{II}$ phase transition, which decreased from 95.2°C to 78.8°C with increasing amounts of water. Above 14.8 %H_2O two transitions were seen which stabilized at 67.1°C and 92.3°C at 19.4 %H_2O. X-ray diffraction data at full hydration confirmed these to be $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$ transitions respectively. Swelling experiments at 23°C showed a lamellar repeat which reached a limiting value at 18 %H_2O in agreement with the DSC data.

Mixed bilayers of DHPE and DPPE at full hydration exhibited two reversible transitions by DSC. X-ray diffraction at 9 mol% DPPE identified these as $L_\beta \rightarrow L_\alpha$ and $L_\alpha \rightarrow H_{II}$. The ether-linkage apparently stabilizes the L_β and H_{II} phases and destabilizes the L_α phase since addition of DHPE to DPPE bilayers increased the $L_\beta \rightarrow L_\alpha$ transition slightly from 63.9°C and decreased the $L_\alpha \rightarrow H_{II}$ transition from an estimated 118°C for pure DPPE.

W-Pos223

EFFECTS OF PROGRESSIVE METHYLATION OF THE PHOSPHATIDYLETHANOLAMINE HEADGROUP (PX) ON THE MIXING BEHAVIOR OF C(22):C(12)PC/C(17):C(17)PX BINARY MIXTURES IN THE BILAYER. R. B. Sisk and C. Huang, Dept. of Biochemistry, University of Virginia, Charlottesville, Va 22908.

The thermotropic phase behavior of aqueous dispersions of binary mixtures of C(22):C(12)PC with diC(17)PC, diC(17)PE$(CH_3)_2$ or diC(17)PE(CH_3) at varying molar ratios was investigated by high-resolution differential scanning calorimetry. C(22):C(12)PC packs in a mixed interdigitated mode at $T < T_m$ and in a partially interdigitated mode at $T > T_m$. In the liquid-crystalline phase ($T > T_m$) C(22):C(12)PC bilayers are about equal in thickness to diC(17)PX bilayers. At $T < T_m$ diC(17)PX packs in a slightly interdigitated mode with a bilayer width about 1/3 greater in thickness than C(22):C(12)PC. Thus C(22):C(12)PC and diC(17)PX mixtures at $T < T_m$ display different packing characteristics and appear to be phase separated, and at $T > T_m$ display similar packing characteristics and appear to be miscible to varying degrees with respect to the PX head group component. Phase diagrams for these binary mixtures exhibit the characteristic shape typical of a eutectic system. Progressively changing the head group of diC(17)PX from PC to monomethylPE has no significant effect on the eutectic horizontal temperature but reduces the mole percent of the diC(17)PX component at which the eutectic point occurs. As a result the $L+G_1$ region of the phase diagrams is progressively reduced in size and the size of the $L+G_2$ region is concomitantly increased. Changes in these regions reflect the relative difference in the immiscibility of the component lipids in the two-dimensional plane of the bilayer as caused by the modification of the lipid headgroup. Supported by USPHS Grant GM-17452.

W-Pos224

THE ROD TO VESICLE TRANSITION IN BILE SALT-LECITHIN MIXED AQUEOUS COLLOIDS Rex P. Hjelm, Jr.[1] P. Thiyagarajan[2], Devinderjit S. Sivia[1], Peter Lindner[3], Hayat Alkan[4], and Dietmar Schwahn[5]. [1]LANSCE, H805, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, USA; [2]IPNS Division, Argonne National Laboratory, Argonne, Illinois 60139, USA; [3]Institut Laue-Langevin, PB 156X-38042, Grenoble CEDEX, France; [4]Department of Pharmaceutics, University of Illinois, Chicago, Illinois 60612, USA; [5]IFF, Kernforschungsanlage, Postfach 1913, D-5170, Jülich/KFA, FRG.

The concentration-induced and thermally-induced transitions from rod like forms to vesicles in mixed aqueous colloids of the bile salt, glycocholate, and lecithin are studied using small-angle neutron scattering. The concentration-induced formation of rods occurs by aggregation of small, globular mixed micelles into large linear aggregates. Measurements on shear-oriented samples put an upper limit on the micelle lengths. Extended networks of rods are observed as a transitional form between rods and vesicles. Vesicles formed at the highest concentrations are quite large, and decrease in size as the total lipid concentration is lowered. All vesicles appear to consist of single lipid bilayers. The thermally-induced transition shows the extended networks as intermediate forms. The transition is reversible.

W-Pos225

IR-ATR POLARIZATION MEASUREMENTS OF SPECIFICALLY LABELED, ORIENTED PHOSPHOLIPID MULTILAYERS.

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IR-ATR spectra of oriented multilayers of specifically ^{13}C=O labeled phospholipids [2,(1-^{13}C) DMPC and 2,(1-^{13}C) DMPA] were measured and analyzed. The orientation of the phospholipid molecules was determined by means of polarization measurements; the results are in general agreement with earlier measurements performed by Fringeli (Z. Naturforsch 32c 20-45, 1977). The use of these isotopically labeled lipids has further clarified several aspects such as the orientation of the two carbonyl groups in the dry state and in the hydrated state below and above the main phase transition temperature. The effect, of the incorporation of cholesterol or of a saturated hydrocarbon, on the tilt angle of the lipid acyl chains and on the orientation of the polar head groups, has also been investigated.

W-Pos226

EFFECT OF α-LINOLENIC ACID AND γ-LINOLENIC ACID ON PHOSPHATIDYLCHOLINE BILAYERS.

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A comparison is made between the 18-carbon fatty acids, α-linolenic (18:3$^{\Delta 9,12,15}$, omega-3) and γ-linolenic (18:3$^{\Delta 6,9,12}$, omega-6) as they affect the properties of phospholipid bilayer membrane fluidity, permeability and fusion. The fatty acids were incorporated into the bilayers as either free fatty acids or as components of synthetic, mixed acyl chain phosphatidylcholines (PC's). The mixed chain PC's were 18:0, α-18:3 PC and 18:0, γ-18:3 PC. Fluidity was determined by fluorescence polarization of a series of anthracene stearic acid probes, permeability by multilamellar vesicle swelling in isomolar erythritol and fusion by fluorescence resonance energy transfer. It is concluded that these two fatty acids affect the fusogenic and permeability properties of PC bilayers to a different extent, with α-linolenic affecting the processes much more than γ-linolenic acid. In contrast both fatty acids similarly affect bilayer fluidity.

W-Pos227

INTERACTION OF S100b PROTEIN WITH MODEL MEMBRANES.

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S100b is the ββ isoform of S100 proteins, a group of three closely related Ca^{+2} binding proteins, structurally related to Ca^{+2} binding proteins of the EF hand type. S100b is abundant in the central and peripheral nervous system, in adipocytes and in melanocytes.

We have previously shown that S100 interacts with model and natural membranes. Both the physical state and the protein structure are modified following the interaction. Specific behaviours are observed in the presence of negatively charged cardiolipin, which could be related to the modification of the lipid membrane structure.

W-Pos228

ATTENUATED TOTAL REFLECTION SPECTROSCOPY OF BILAYERS AND MEMBRANES COATED ON INFRARED OPTICAL FIBERS.

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Lipid and lipid/protein systems have been investigated using a newly developed IR optical fiber attenuated total reflectance (OFATR) technique. Using simple film casting/dipping techniques, lecithin bilayers (with or without acetylcholine receptor) were applied to the surface of the OFATR element. The single-dip (bilayer) films on the optical fiber were extremely stable, tolerating multiple washes without detectable IR absorbance changes. FTIR spectra could thus be obtained of membrane samples submerged in buffer at atmospheric pressure. Spectra obtained using the OFATR apparatus should be more representative of physiological membrane systems than previous IR spectra of membranes, which generally utilized partially dried samples pressed between windows or cast as films on the surface of bulk ATR elements. The immobility of the membranes on the OFATR element allows for rapid buffer changes without disrupting the sample. Furthermore, measurements were made on films consisting of very small amounts of material (~1 nmol lipid and ~3 pmol protein). These experiments demonstrate the advantages of using the OFATR method to study membrane systems.

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W-Pos229

THE EFFECT OF UNSATURATED PHOSPHATIDYL-ETHANOLAMINE ON THE CHAIN ORDER PROFILE OF BILAYERS AT THE ONSET OF HEXAGONAL PHASE TRANSITION. D.B. Fenske and H.C. Jarrell, Division of Biological Sciences, NRC, Ottawa, Canada K1A 0R6, Y. Guo and S.W. Hui, Roswell Park Memorial Institute, Buffalo, New York 14263.

The order parameter profile of methylene groups along the acyl chains of mixtures of dioleoyl phosphatidylethanolamine (PE) and dimyristoyl phosphatidylcholine (PC) at the onset of the lamellar to hexagonal phase transition of the mixtures was investigated by ^{2}H-NMR. The quadrupolar splittings and the corresponding probability of gauche isomeric state at various carbon positions along the chain were measured as functions of temperature and PE/PC ratio. The sensitivity of the quadrupolar splitting increases with decending carbon numbers. Small angle X-ray diffraction showed decreasing trend in area per molecule with increasing PE percentage. The chain motion profiles at increasing PE percentage gives a measure of differential lateral pressure experienced along the length of the chains. A molecular packing model is proposed to explain our observations.

W-Pos230

PHOSPHATIDYLETHANOLAMINE MELTING VOLUMES

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We have determined the volumes of melting of the saturated phosphatidylethanolamines DLPE, DMPE, DPPE and DSPE using high sensitivity differential scanning calorimetry under variable hydrostatic pressure and the Clausius-Claperyon equation. The melting volumes of DLPE and DMPE were 0.01830 and 0.02256 mL/gm respectively, as contrasted with 0.01600 and 0.02040 mL/gm reported by Wilkinson and Nagle on the basis of dilatometric experiments. The volumes for the calorimetric experiments are apparently larger than for the dilatometric experiments because the latter were carried out under conditions which allowed partial dehydration of the phosphatidylethanolamines to form unhydrated crystalline phases. The melting volumes of 0.03019 and 0.04604 mL/gm which we found for DPPE and DSPE reflect the fact that the volume of melting per CH_2 increases with chain length, as for the phosphatidylcholines. The relative increase is greater for the phosphatidyl-ethanolamines, however. This work was supported by a grant from the American Registry of Pathology and by NIH grant GM40155.

W-Pos231

THE ROLE OF CHAIN ORDERING IN THE FORMATION OF LIPID BASED TUBULES

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Molecules of DC89PC, a diacetylenic phospholipid, self-organize in the presence of a solvent into hollow crystalline cylinders called "tubules". In order to elucidate the role played by chain order in tubule formation, we have undertaken a FTIR study of DC89PC dispersed in different volume fractions of ethanol-water. No significant differences are observed in the intrachain order of the tubular and non-tubular solid forms of DC89PC. However, pronounced differences with regards to both stretching and bending modes are seen in the higher temperature disordered phase, these changes being functions of the ethanol fraction. Additional data on the high temperature disordered phase and its significance in the formation of tubules will be presented.

W-Pos232

A DIRECT MEASUREMENT OF THE DIFFUSE DOUBLE LAYER AT A MEMBRANE/AQUEOUS INTERFACE USING LONG-PERIOD X-RAY STANDING WAVES.

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Ion distribution profile in an electrolyte solution in contact with a charged polymerized phospholipid monolayer was directly measured with long-period x-ray standing waves. The 27Å thick membrane was supported on a tungsten/silicon mirror which was used to generate the standing waves by means of total external reflection. The membrane surface contained the negatively charged phosphate headgroups and was bathed in a dilute $ZnCl_2$ solution. Zn^{2+} distribution in the diffuse double layer and Zn^{2+} binding at the membrane surface were measured as a function of headgroup charge and were found to follow the Gouy-Chapman-Stern theory.

W-Pos233

INCORPORATION OF MYELIN PROTEINS INTO A MULTILAMELLAR MEMBRANE-LIKE ENVIRONMENT : MODIFICATION OF LAMELLAR PHYSICAL PROPERTIES

D. CHATENAY, D. LANGEVIN, A. OTT and W. URBACH, ENS 75231 PARIS CEDEX 05 - C. NICOT, M. WAKS, UER BIOMEDICALE DES SAINTS PERES 75270 PARIS CEDEX 06 - R. OBER, COLLEGE DE FRANCE 75231 PARIS CEDEX 05.

Properly oriented multilamellar phases can be prepared by mixing a cationic surfactant dodecyltrimethyl ammonium bromide (DTAB) with well defined quantities of brine and pentanol. Physical properties of the system have been measured by both X-ray and quasi-elastic light scattering (QELS). The two major myelin membrane proteins have been inserted into the multilayers and characterized by spectroscopic studies (absorption and fluorescence). Preliminary results indicate a significant change in the rigidity and compressibility of the bilayers induced by the presence of the protein. Synthetic lamellar systems may thus provide further insights into the interaction of myelin proteins with biological multilamellar membranes.

W-Pos234

^{2}H NMR STUDIES OF A STRUCTURAL ROLE FOR VITAMIN E IN MEMBRANES. Stephen R. Wassall[1], M. Alan McCabe[1], William Ehringer[2] and William Stillwell[2], Departments of Physics[1] and Biology[2], Indiana University-Purdue University at Indianapolis, Indianapolis, IN 46205.

The proposal that α-tocopherol, the major constituent of vitamin E, can stabilize membranes by forming complexes with free unsaturated fatty acids was investigated by broadline ^{2}H NMR. Spectra for *sn*-1,2-[$^2H_{62}$]DPPC membranes containing stearic (18:0) or linoleic (18:2) acids were recorded, and the effects of the introduction of α-tocopherol were compared. Moment analysis confirms that the two fatty acids affect membrane phase behaviour differently, but provides no evidence for stabilization of membrane structure in the presence of α-tocopherol. ^{2}H NMR of deuterated stearic and linoleic acids further tests the hypothesis.

W-Pos235

LATERAL DIFFUSION AND PHASE DIAGRAMS OF POLYMERIZED LIPID BILAYER MEMBRANES. D.A. Pink, E.Sackmann, B.E.Quinn, Theoretical Physics, St. Francis Xavier University, Antigonish, NS, Canada and Dept.-Physik, Technischen Universitat Munchen, D-8046 Garching, F.R.G. We have modelled a lipid bilayer membrane composed of a linearly polymerized amphiphile, with the molecules interconnected via the headgroups, and dimyristoylphosphatidylcholine (DMPC). We have performed Monte Carlo simulations in order to calculate (a) the phase diagram of this quasi-two dimensional system and (b) the dependence of the lateral diffusion coefficients of both DMPC and the polymers upon DMPC concentration for temperatures sufficiently above T_c (DMPC). Comparisons will be made with the experimental data of Eggl et al. (to be published). Work supported by NSERC and D.F.G.

W-Pos236

STATISTICAL MECHANICS OF THE RIPPLE PHASE IN LIPID BILAYERS. W. S. McCullough and H.L. Scott (Intro. by R. Floyd), Department of Physics, Oklahoma State University, Stillwater, Ok. 74078.
We present the results of analytical and computer studies of a lattice model for lipid bilayers below the main lipid phase transition temperature. The model consists of a two dimensional lattice occupied by block "L"shaped molecules which represent lipids with protruding head groups and with chain disordering frozen out. Each molecule may move perpendicular to the layer plane, and the L-molecules may orient in one of two directions along an axis in the layer. Interaction energies calculated by Pearce and Scott can be mapped onto a Hamiltonian function and the phase properties determined using Statistical Mechanics. In this paper we present the results of analytical and computer studies of the model. We have found that the model exhibits a modulated phase only if the relative differences in the height variable between neighboring molecules is restricted to a certain range, and even in this case standard mean field theory fails to predict the ripple phase. With the height restrictions the model more closely resembles a class of models known as Restricted Solid-on-Solid (RSOS) Models. With this result we analytically estimate the phase properties of the model, and compare with experimental data and Monte Carlo simulations. The model predicts a chain-tilted low temperature phase followed by a ripple phase followed by a disordered phase. (Supported in part by NSF Grant No. DMR-8703644)

W-Pos237

CALORIMETRIC DETECTION OF THE LAMELLAR/ INVERTED CUBIC (Q_{II}) PHASE TRANSITION IN N-MONOMETHYLATED DIOLEOYLPHOSPHATIDYL-ETHANOLAMINE (DOPE-Me)

D. P. Siegel, J. L. Banschbach -- Procter & Gamble Co., P.O. Box 398707, Cincinnati, OH 45239-8707

We have calorimetrically detected the L_α/Q_{II} phase transition in DOPE-Me at temperature scan rates of 1°C/hour. Together with our previous X-ray diffraction data [1], the results indicate that a Q_{II} phase of the Pn3m space group is thermodynamically stable in the temperature range beginning at the equilibrium L_α/Q_{II} transition temperature of 61 ± 1°C. Additional broad endotherms are sometimes detected at $T \geq 72$°C, which may be due to Q_{II}/H_{II} transitions. The L_α/Q_{II} endotherms can be as narrow as 1°, but are often wider. Even at a scan rate of 1°C/hour, L_α/Q_{II} endotherms sometimes occur at temperatures up to 63°C, consistent with the transition time of hours deduced previously [1]. The enthalpy of the L_α/Q_{II} transition is ca. 60% of the enthalpy of the L_α/H_{II} transition (observed at 66.1 ± 0.7°C with scan rates $\geq$13°C/hr). These results will be discussed in the context of theoretical models of Q_{II} phase stability and formation mechanisms. --- [1] Biophys. J. 55:28a.

W-Pos238

ALCOHOL CHAIN LENGTH EFFECTS IN THE ALCOHOL INDUCTION OF INTERDIGITATION IN DISTEAROYLPHOSPHATIDYLCHOLINE.

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The saturated like-chain phosphatidylcholines (PC's) exist in a fully interdigitated gel phase in the presence of various amphipaths including ethanol. We have previously demonstrated the detection of the transition of PC's from the non-interdigitated (L_β') to the interdigitated ($L_\beta I$) bilayer as a function of temperature and ethanol concentration by the fluorescence intensity of diphenyhexatriene (DPH)[Nambi, P., McIntosh, T.J.,and Rowe, E.S.(1988) Biochemistry 27:9175-9182]. We now report our further characterization of the use of fluorescence methodology to detect interdigitation by investigating the main transition and distinguishing between the L_β' to L_α and the $L_\beta I$ to L_α transitions. Using this improved fluorescence method we have investigated the effect of the alcohol chain length on the concentration and temperature dependence of the alcohol induction of interdigitation in distearoylphosphatidylcholine (DSPC). (Supported by the NIAAA and the Veterans Administration.)

W-Pos239

STABILIZATION OF LIPID BILAYER VESICLES DURING FREEZE/THAWING BY SINGLE-CHAIN DETERGENTS.

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Dynamic light scattering, giving bimodal size distributions, and release of vesicle-entrapped carboxyfluorescein showed that freeze-induced aggregation and leakage of small unilamellar phosphatidyl serine and phosphatidyl choline vesicles is suppressed by sodium dodecylsulfate and cetyltrimethyl ammonium chloride. At 1-3% detergent:lipid mole ratios all or most of the lipid remained as small (25-40 nm) vesicles after freezing and subsequent thawing, with only little loss of entrapped solute. Without detergent, freezing produced aggregates of ca. 2000 nm and extensive solute loss. Lysolecithin gave only limited freeze-protection. Equilibration of lipid with detergent for 24 hours prior to freezing was required to obtain this stabilizing effect. It is concluded that the detergents must interdigitate with the lipid bilayers for stabilization to occur. This is yet another example of the stabilization of vesicles by alteration of the effective headgroup area, as also observed with saccharides.

W-Pos240

PHASE BEHAVIOUR OF DIACYLGLYCEROL MIXTURES WITH PHOSPHATIDYL CHOLINES AND PHOSPHATIDYL-GLYCEROLS.

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(Intro. by J.K. Zimmerman)
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The binary phase diagrams of phosphatidyl choline-diacyl glycerol mixtures, where both components have either 1, 2-dimyristoyl chains (DMPC:DMG), 1, 2-dioleoyl (DOPC:DOG) chains, or 1-stearoyl-2-oleoyl chains (SOPC: SOG), and of phosphatidyl glycerol-diacyl glycerol mixtures, where both components have 1, 2-dimyristoyl chains (DMPG:DMG), have been studied by differential scanning calorimetry, spin label ESR spectroscopy, ^{31}P NMR spectroscopy and x-ray diffraction. The phase diagram of DMPC:DMG mixtures displays three regions corresponding to the existence of compounds (C1 and C2, respectively) with approximately 1:1 and 1:2 mole/mole DMPC: DMG stoichiometries. The first region displays immiscibility between DMPC and C1 in the low temperature lamellar phase and miscibility of the components in the fluid phase which is lamellar. The second region displays immiscibility between C1 and C2 in the low temperature phase which is lamellar, whereas the fluid phase is of the inverted hexagonal type (H_{II}). The third region displays immiscibility between C2 and DMG in the low temperature phase which is lamellar, whereas the fluid phase is isotropic. The phase diagram of DMPG:DMG at low ionic strength differs from that of DMPC:DMG in that the compound C3 is no longer found and the high temperature region at DMG compositions greater than equimolar is a mixture of (immiscible) fluid lamellar and isotropic phases. The H_{II} phase is totally suppressed, indicating the important influence of electrostatic interactions on the phase and mixing behaviour.

W-Pos241

THE SURFACE TENSION OF MONOLAYERS FORMED OVER LIPID VESICLE DISPERSIONS

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The surface tension at the air/water interface of a dispersion of phospholipid vesicles falls with time and eventually reaches an equilibrium value as a monolayer is deposited on the surface. To understand the mechanism of formation of such monolayers, we have been studying their rate of formation under a variety of conditions. The experimental procedure involves measuring the surface pressure of a vesicle suspension in a small trough; the surface is swept clean of monolayer and flux to the surface during return to equilibrium is monitored as a change in surface tension by the Wilhelmy method. Transport rates from bilayer to surface depend upon conditions in unexpected ways. For a given vesicle concentration, sonication reduces the rate of monolayer formation. Since sonication must increase the number of vesicles, it must increase the barrier to transfer of lipid to the forming monolayer. In addition, bilayer to monolayer transfer rates are highly dependent on concentration and, for ionic lipids, on electrolyte concentration. Thus, under some conditions, sonication may so delay equilibration that sonicated vesicles may appear to have unusually low equilibrium tensions. Large MLVs behave oppositely to small vesicles with respect to the concentration of electrolyte; MLVs equilibrate slower, SUVs faster, as electrolyte concentration is raised. Supported by GM38244.

W-Pos242

THE EFFECT OF HIGH PRESSURE ON MEMBRANE SURFACE CHARGE AS DETERMINED BY pH-SENSITIVE PROBES

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We have investigated the effect of pressure on membrane surface charge by spectroscopically monitoring the dissociation state of two probes (6-decanoyl-naphthol (DECNA) and 3-hexadecanoyl-coumarin (HEXCO)). These probes incorporate into micelles and bilayers. pH titrations show that the head groups lie on the surface. While the charge of the particular micelle changes the dissociation of the probe, these effects are greatly diminished in bilayers. With free probes, high pressure promotes dissociation to the anion due to the condensation of water around the exposed charges (electrostriction). In micelles this mechanism is operative. In bilayers, increasing the surface charge reverses electrostriction effects due to repulsion and limited surface expansion. A linear relationship exists between the initial degree of probe dissociation and the volume change for both micelles and bilayers. Supported by N.I.H. GM39924.

W-Pos243

TEMPERATURE-JUMP INDUCED PHASE TRANSITIONS ON LIPID BILAYERS SHOW HITHERTO UNKNOWN INTERMEDIATES AS MEASURED BY TIME-RESOLVED X-RAY DIFFRACTION

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To investigate the thermotropic phase transitions of phospholipid/water systems with millisecond time resolution we used an erbium laser (1540nm, 2J/pulse, pulse length 2ms) as a fast heating source in combination with small-angle X-ray powder diffraction using synchrotron radiation. Partial absorption of the laser energy raised the temperature approximately 10°. The resulting temperature in the samples (phosphatidyl-ethanolamines, SOPE and dipalmitoyl-lecithin, DPPC) was calculated to be uniform within more than 80%. A fast X-ray detection and data acquisition system allowed monitoring of the structural changes within 0.5 ms. The results show the existence of short-lived intermediate structures in transitions between phases of different symmetry (pretransition $L_{\beta'}$ - $P_{\beta'}$ in DPPC and L_α - H_{II} transition in SOPE). Lamellar/lamellar transitions as e.g. the L_β - L_α transition in SOPE occur within less than 3ms, suggesting a strongly correlated mechanism. These measurements are hitherto the highest time-resolved X-ray diffraction experiments on lipid phase transition.

W-Pos244

CHAPS-INDUCED CURRENT FLUCTUATIONS IN PLANAR LIPID BILAYERS. R. V. Jones, E. Rousseau and G. Meissner (Intro. by B. Pallotta). Dept. of Biochemistry, Univ. of North Carolina, Chapel Hill, NC 27599-7260.

The process of purifying and reconstituting transport membrane proteins generally involves the use of detergents which often cannot be completely separated from the proteins. The effects of the detergent CHAPS on planar lipid bilayers have been measured, and it is demonstrated that CHAPS can induce microscopic electrical activity in the bilayers. The probability of obtaining such activity was enhanced by increasing the CHAPS concentration or by increasing the holding potential. Typical CHAPS-induced activity consisted of large current bursts, often separated by intervals of quiescent activity, with no definable conductance levels. The size of the current bursts was generally increased by nM free Ca^{2+} and mM ATP and reduced by mM Mg^{2+} and μM ruthenium red. Supported by NIH.

W-Pos245

LIPOSOME RADIUS AFFECTS THE CHOLESTEROL INHIBITION OF CYT B_5 INSERTION. Kenneth M.P. Taylor and Mark A. Roseman, Dept. of Biochem., Uniformed Services Univ., 4301 Jones Bridge Rd., Bethesda, MD 20814

Cholesterol has been implicated in preventing cyt b_5 from binding to plasma membranes[1]. This is apparently contradicted by the observation that cyt b_5 binds to egg PC SUVs containing 44 mole percent cholesterol[2]. To resolve this discrepancy we have examined cyt b_5 interaction with LUVs and SUVs of 1-palmitoyl-2-oleoyl PC containing 50 mole percent cholesterol. In direct binding mixtures (1:400, cyt b_5:PC), >94% of cyt b_5 binds to SUVs, whereas <5% binds to LUVs. These results show that cholesterol inhibits cyt b_5 partitioning into bilayers. However, the radius of curvature of liposomes has a larger effect on cyt b_5 binding from the aqueous phase than expected. LUVs are probably a more valid model system for plasma membrane studies. If so, these liposome studies are consistent with the proposal that cholesterol does inhibit cyt b_5 binding to plasma membranes. [NIH Grant DK30432]

[1]Enomoto, K. and Sato, R. Biochim. Biophys. Acta 466, 136-147 (1977); [2]Roseman et al, Biochim. Biophys. Acta 507, 552-556 (1978).

W-Pos246

Cytochrome b_5 Association with DMPC Bilayers. Chester, D., Young, H., Skita, V., Strittmatter, P. Department of Biochemistry and Biomolecular Structure Analysis Center University of Connecticut Health Center, Farmington, CT. 06032

The nonpolar peptide (NPP) portion of cytochrome b_5 serves to anchor this protein in the membrane bilayer. Previous attempts by this lab and others to examine the low resolution membrane bound cytochrome b_5 structure were unable to unambiguously determine NPP orientation and bulk distribution within the bilayer. We are attempting to determine, at high resolution, the structure of DMPC membranes containing asymmetrically reconstituted b_5. To a first approximation, we have obtained phases for the data by Patterson function deconvolution. These phases were then used to Fourier reconstruct the electron density profile to 8Å resolution. Preliminary calorimetry results suggest that b_5 incorporation into the bilayer at high concentration appears to broaden the thermal phase transition both above and below Tm for pure DMPC. These studies supported by NIH GM-15924.

W-Pos247

CRYO-TEM REVEALS STRUCTURAL TRANSITIONS OF EGG PC AND SODIUM CHOLATE MIXTURES. **A Walter, PK Vinson & Y Talmon. Wright State Univ, Dayton, OH; Univ Minnesota, Minneapolis, MN; Technion, Haifa, Israel.**

The vesicle-micelle transition of egg PC and sodium cholate in dilute solution was examined by cryo-transmission electron microscopy (TEM), a technique that permits direct observation of unstained, vitrified hydrated samples. Cryo-TEM samples were prepared from extruded egg PC vesicles and varying concentrations of cholate at pH 7.2 that corresponded to specific changes in vesicle integrity, in the phospholipid interactions and in solution OD. At very low [cholate], MLV and small vesicles (20-30 nm dia) were seen from an original preparation of LUV. As cholate was added, open vesicles and long rod-shaped micelles were found in co-existence until all the lipid was in mixed micelles. At higher [cholate], the rods were shorter, and some spheroidal micelles (<5 nm) were observed. At clarity, only spheroidal micelles were present. No discoidal structures were seen. These transition structures match those seen with egg PC and octyl glucoside, although the surfactant-to-phospholipid ratios were quite different.

W-Pos248

RECONSTITUTION OF THE Na^+-SELECTIVE Na^+/H^+ ANTIPORTER FROM BEEF HEART MITOCHONDRIA: QUANTITATION OF Na^+ TRANSPORT WITH THE NOVEL FLUORESCENT PROBE, SBFI. Sati Nath, Petr Jezek and Keith D. Garlid, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

The novel fluorescent probe, SBFI was used to assay Na^+ transport in proteoliposomes reconstituted from protein extracts of beef heart mitochondria in the presence of detergent and lipids. Study of reconstituted crude extracts was complicated by the presence of two Na^+/H^+ antiporters, so we removed the DCCD-sensitive K^+/H^+ (and Na^+/H^+) antiporter by a single chromatographic step. In agreement with previous studies by us and others on intact mitochondria, reconstituted Na^+/H^+ antiport was inhibited by Li^+ and Mn^{2+} and with similar K_i values. Na^+ efflux on the exchanger was also inhibited by external Li^+, but the K_i (25 mM) was much elevated. No covalent inhibitor is known for the Na^+-selective Na^+/H^+ antiporter, and reconstitution is the only method available for tracking the carrier during fractionations. Our findings will now be used to develop an isolation procedure for this elusive carrier, which is as "old" as the chemiosmotic theory. This research was supported by NIH Grants GM 31086 and HL 36573.

W-Pos249

RECONSTITUTION OF THE K^+/H^+ (Na^+/H^+) ANTIPORTER FROM BEEF HEART MITOCHONDRIA: QUANTITATION OF K^+ TRANSPORT WITH THE NOVEL FLUORESCENT PROBE, PBFI. Keith D. Garlid, Mohammed Hegazy, Petr Jezek, and Fakhri Mahdi, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

The novel fluorescent probe, PBFI, provides a convenient and sensitive assay for the study of K^+ transport mediated by the reconstituted K^+/H^+ (Na^+/H^+) antiporter. PBFI fluorescence has enabled us to establish the first successful reconstitution of K^+/H^+ antiport activity from beef heart mitochondria. We show that propranolol and timolol inhibit reconstituted K^+/H^+ exchange with I_{50}s similar to those observed in intact mitochondria. We show that dicyclohexylcarbodiimide (DCCD) is capable of complete inhibition of K^+/H^+ antiport in the reconstituted system, in accord with findings in intact mitochondria. PBFI fluorescence, which measures net K^+ uptake, was essential for this corroboration, since DCCD is not capable of complete inhibition of $^{42}K^+/K^+$ or $^{86}Rb^+/Rb^+$ exchange, presumably because it acts selectively on proton transport within the carrier. A newly purified beef heart K^+/H^+ antiporter protein is also shown to be reconstitutively active using PBFI. This research was supported by NIH Grants GM 31086 and HL 36573.

W-Pos250

BILAYER ASSEMBLY IN NEURAL MEMBRANES IS A CRITICAL PHENOMENON.
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Cell membrane bilayers have been reconstructed in vitro utilizing total lipid extracts from rat neural tissue (forebrain, cerebellum, brainstem and spinal cord) and from the optic lobe and fin nerve of the squid Loligo pealei. In agreement with the critical state theory of bilayer assembly (1,2), these lipid extracts spontaneously formed purely unilamellar structures in aqueous dispersion, but only at a critical temperature, T*, which was species-dependent. For all the rat tissues T* = 37±1°C, for squid neural extracts T* = 15±1°C. These values correspond to 'physiological' temperatures for both organisms, implying that their lipid metabolism is geared to permit spontaneous assembly of unilamellar membranes at the ambient temperature in the tissues. Membrane proteins had little or no effect on critical bilayer formation.

1. Gershfeld, N. L. (1986) Biophys. J. 50, 457-461;
2. Gershfeld, N. L. (1989) J. Phys. Chem. 93, 5256-5261.

W-Pos251

EFFECT OF PHOSPHOLIPID FATTY ACYL COMPOSITION ON THE SPONTANEOUS INCORPORATION OF BACTERIORHODOPSIN.
Anthony W. Scotto, Dept. of Medicine, Cornell University Medical College, New York, NY 10021

Bacteriorhodopsin spontaneously incorporated into either fluid or gel phase small preformed unilamellar vesicles (SUVs) comprised of phosphatidylcholine regardless of the fatty acid composition. Bacteriorhodopsin also spontaneously incorporated into large unilamellar vesicles (LUVs)(0.05-0.1μm) comprised of either dimyristoyl- or dioleoylphosphatidylcholine (PC) and the incorporation of this membrane protein was not effected by the addition of cholesterol. Bacteriorhodopsin did not spontaneously incorporate into fluid phase LUVs of either 1-palmitoyl-2-oleoyl-PC (POPC) or 1-oleoyl-2-palmitoyl-PC (OPPC). However, LUVs prepared from mixtures of either POPC and OPPC or POPC and cholesterol did spontaneously incorporate bacteriorhodopsin. Thus, the only preformed vesicles found to be not receptive to the spontaneous incorporation of bacteriorhodopsin are LUVs prepared of pure POPC. The significance of these findings to spontaneous insertion events that may play a role in natural membranes will be discussed. (Supported by NIH grant GM 36651).

W-Pos252

TRIMERIC GATING OF BACTERIAL PORINS RECONSTITUTED IN LIPID BILAYERS

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Voltage-dependent gating is demonstrated for homo- and heterotrimers of the bacterial porins PhoE, OmpF, OmpC, LamB, and OmpF/OmpC incorporated into PE/PC black lipid membranes. The incorporation process depends upon the magnitude and polarity of the applied membrane potential. By varying these conditions, we were able to show how the onset of channel gating depends on the initial conditions of incorporation, and to reproduce and reconcile the conflicting results in the literature about porin gating. For all the porins, individual trimers show voltage gating as cascades of three unitary steps, consistent with the trimer structure of three independent pores suggested by structural studies. The individual monomeric pores gate relatively independently of each other with transition rates that are a very steep function of voltage. The porin channel gating also manifests fast flickering and an additional rapidly flickering subconductance level, as well as a residual closed-state conductance.

W-Pos253

ISOLATION AND RECONSTRUCTION OF A Ca^{2+}-ACTIVATED K^+ CHANNEL FROM BASOLATERAL MEMBRANES OF TRACHEAL EPITHELIAL CELLS.

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Calmodulin-affinity chromatography was employed to isolate a Ca^{2+}-activated K^+ channel from the basolateral membranes of bovine tracheal epithelial cells. These membranes were prepared from epithelial scrapings, solubilized in 3.7% CHAPS and centrifuged at 100,000 x g for 40 min. to remove particulate matter. The soluble fraction was applied to a calmodulin-Sepharose 4B affinity column equilibrated with detergent and 100 μM Ca^{2+}. After washing in the presence of Ca^{2+}, the calmodulin-bound proteins were eluted by washing with a Ca^{2+}-free buffer. K^+ channel activity was detected following the reconstitution of this eluate into planar phospholipid bilayers in the presence of 10 μM Ca^{2+} and 1 μg calmodulin. This activity was abolished by reducing the Ca^{2+} activity to < 10^{-8} M and restored by elevating that activity to 1 μM. The current-voltage relation of the channel is linear and, in the presence of 150 mM KCl/50 mM KCl solutions, has a conductance of ~400 pS. [Supported by NIH research grants (DK-38518) and (DK-37620).]

W-Pos254

KINETICS OF Cl^- UNIPORT THROUGH THE RECONSTITUTED, PURIFIED UNCOUPLING PROTEIN (UcP) OF BROWN ADIPOSE TISSUE MITOCHONDRIA (BATM).

David E. Orosz, Petr Jezek and Keith D. Garlid, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

The fluorescent chloride indicator, SPQ, was trapped in proteoliposomes reconstituted with purified 32 kDa **UcP** and used to detect GDP-sensitive uniport of Cl^-, Br^-, I^-, and (indirectly) F^-. This preparation also exhibited $H^+(OH^-)$ transport, showing that **UcP** possesses both halide and H^+ transport functions. Cl^- transport was inhibited 50% by 1 mM external GDP and 100% when 1 mM GDP was present on both sides of the membrane, indicating equal sidedness distribution of reconstituted **UcP**. The K_ms for Cl^- and Br^- uniport were ca. 65 and 80 mM, resp. V_{max} was independent of pH. Mersalyl inhibited $H^+(OH^-)$ transport but not Cl^- uniport, in agreement with findings in **BATM**. Cl^- uniport depends nonohmically on $\Delta\Psi$ with parameters different from ion leak and suggesting an energy well (i.e., low affinity binding site) in the **UcP** Cl^- pathway located near the center of the membrane. Thus, we have characterized a Cl^- translocation mechanism that was previously reported as lacking in the reconstituted system. This research was supported by NIH Grant GM 31086.

W-Pos255

ALKYLSULFONATES - A NEW CLASS OF COMPETITIVE INHIBITORS OF CHLORIDE UNIPORT THROUGH THE RECONSTITUTED, PURIFIED UNCOUPLING PROTEIN (UcP) OF BROWN ADIPOSE TISSUE MITOCHONDRIA (BATM).

Petr Jezek, David E. Orosz, and Keith D. Garlid, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

We have identified a new series of anionic substrates for the **UcP** of **BATM** that also exhibit chain-length-dependent competitive inhibition of Cl^- uniport through **UcP**. These properties were observed in a reconstituted system using the fluorescent indicator, SPQ, to detect Cl^- uptake and confirmed in intact **BATM** using light scattering technique. The entire series, methane- through hexanesulfonate, exhibits GDP-sensitive transport and inhibition of Cl^- transport, both increasing strongly with alkyl chain length. Hexanesulfonate increases the K_m but does not affect the V_{max} of Cl^- uniport, characteristic of competitive inhibition. These surprising results showing that **UcP** is able to transport large substrates support and extend a hypothesis of common transport mechanisms in the carrier family including **UcP**, the P_i/OH^- antiporter and the ADP/ATP translocator. This research was supported by NIH Grant GM 31086.

W-Pos256

EFFECT OF SURFACE ADSORPTION OF MOLECULES UPON TWO-DIMENSIONAL TRACER DIFFUSION. J.R. Powell, D.A.Pink, B.E.Quinn, Theoretical Physics, St. Francis Xavier University, Antigonish, NS, Canada. We have modelled the movement of a probe in the plane of a surface upon which molecules, e.g. proteins, are adsorbing/desorbing from/to a bulk reservoir thereby changing probe movement via steric interactions. The intention is to see what information can be obtained about the molecules, such as residence time at the surface and binding energies, from a knowledge of tracer diffusion. We have carried out a Monte Carlo simulation of this model and will report results for the diffusion coefficient as a function of these parameters and bulk molecule concentration. We will also report on the critical exponents for such diffusion when the surface concentration is at the static percolation limit. Work supported by NSERC of Canada

W-Pos257

EFFECTS OF LATERAL DIFFUSION ON THE FLUORESCENCE ANISOTROPY IN THE HEXAGONAL H_{II} PHASE.

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The P_2P_4 model for Fluorescence Anisotropy in bilayers [Biophys.J. 46 (84) 515] has been extended to include the effects of lateral diffusion over the lipid-water interface. This interface was assumed to consist of a collection of cylinders with radius R. The preferred orientation of the molecules is perpendicular to the cylinder surfaces, but the axes are isotropically distributed over the sample. A transition to a lamellar phase would correspond to $R \rightarrow \infty$. It is shown that lateral diffusion over the interface gives rise to two new rotational correlation times $T_1 = R^2/D_L$ and $T_2 = T_1/4$, where R is the radius of curvature of the interface and D_L is the lateral diffusion constant. In the hexagonal phase these times are in the 10-100 nanosecond timescale and may cause appreciable depolarization. We have restricted ourselves to probes with absorption and/or emission moments along the long axis of the molecule, which is considered to have effective cylindrical symmetry. Our model predicts a fluorescence anisotropy decay containing 9 exponentials. The rotational correlation times are combinations of T_1 or T_2 and times depending on D_w, the wobbling diffusion constant, and on the orientational order parameters, $\langle P_2 \rangle$ and $\langle P_4 \rangle$. The fluorescence anisotropy is proportional to r_o, the fundamental anisotropy. Using global analysis of fluorescence decay surfaces the simultaneous determination of R^2/D_L, D_w, $\langle P_2 \rangle$, $\langle P_4 \rangle$ and r_o is feasible.

W-Pos258

TIME-RESOLVED FLUORESCENCE STUDY ON THE ROTATIONAL DYNAMICS OF LIPID LAMELLAR (L) AND INVERTED HEXAGONAL (H) PHASES

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The polymorphic phase behavior of unsaturated phosphatidylethanolamine (PE)/diolein binary lipid mixtures was studied by time-resolved fluorescence anisotropy technique [Biophys. J. 55 (89) 1025]. A fluorescent lipid, diphenyl- hexatriene-labeled phosphatidylcholine (DPH-PC), was used to explore the orientational order and rotational dynamics of the above lipid mixtures. Using a 1st order approximation, abrupt changes in both order parameter and rotational correlation time of DPH-PC were observed at 7% diolein, which signified the L/H transition. Using both a 2nd order approximation and a new model (see Van Der Meer et al. abstract), more than one rotational correlation times were required to describe the anisotropy decay behavior of DPH-PC in the H phase. The rotational correlation times were related to the order parameters and the diffusional constants (wobbling and hopping) of the probe. The lateral diffusion constant of lipids in the H phase was also estimated by pyrene excimer formation technique and compared with the hopping diffusion constant (=lateral diffusion constant/R^2; R=radius of the lipid hexagonal tubes) derived above. This radius R was then determined and found to agree with that derived from the X-ray diffraction method.

W-Pos259

DIRECT DETERMINATION OF THE EFFECT OF CHOLESTEROL ON CONFORMATIONAL DISORDER IN DPPC BILAYERS BY INFRARED SPECTROSCOPY

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A direct determination of the percentage of trans and gauche conformers present in the acyl chains of specifically deuterated DPPC has been made using FTIR. The approach takes advantage of the conformational sensitivity of CD_2 rocking mode frequencies. CD_2 rocking frequencies and band areas of 4,4,4',4'-d_4 DPPC (4-d_4 DPPC), (6-d_4 DPPC), (10-d_4 DPPC), and (12-d_4 DPPC) were monitored for use as conformation markers in the presence and absence of cholesterol. Cholesterol reduced the gauche conformer percentages from 20.7 (4-d_4 DPPC) and 32.3 (6-d_4 DPPC) to between 1.6 and 3.2 percent at 48°C. Such ordering was not observed for the 12-d_4 derivative, indicating that the ordering effect of cholesterol is not operative at bilayer depths beyond the rigid sterol nucleus.

W-Pos260

PARTITIONING AND MEMBRANE DISORDERING EFFECTS OF n-ALKANOLS IN PURE PHOSPHATIDYLCHOLINES. Allan Atienza, Robert D. Smith, Susan Dakin, and Martha Sarasua; Department of Surgery, Cleveland Metropolitan General Hospital and Case Western Reserve University, Cleveland, Ohio.

The partitioning and membrane disordering effects of several n-alkanols in phosphatidylcholines (PCs) were characterized as a function of temperature and phospholipid fatty acid chain length (m=12,14,16,18). Partitioning of ^{14}C-labeled alcohols into these PCs and their effects on the fluorescence polarization of diphenylhexatriene (DPH) were measured. The effects of ethanol and butanol on DPH polarization were maximal at the main phase transition of each PC and reflect increased membrane concentrations of the alcohols at this transition. A PC fatty acid chain length preference of 16 was observed in the effects of ethanol and butanol on DPH polarization at the main phase transition.

W-Pos261

FLUORESCENT LIPID MOVEMENT IN HEATED CELLS.

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At 2°C NBD-ceramide concentrates first in the ER, mitochondia and perinuclear region before moving to the golgi (Pagano et al). In 3T3 fibroblasts ceramide movement to the golgi was delayed directly after heating (44°C for 45 min) while, in thermotolerant (TT) cells, movement was accelerated. The later export to the plasma membrane (PM) was also delayed. In contrast at 2°C phosphatidylcholine (PC) first concentrates at the PM. In TT cells both accumulation and retention were enhanced. Immediately after heating, total cellular ceramide fluorescence was reduced while PC related fluorescence increased. However, in TT cells uptake of both these fluorescent lipids was enhanced over two-fold.

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W-Pos262

FLUORESCENCE LIFETIME DISTRIBUTIONS OF DPH FLUOROPHORES IN PHOSPHOLIPID BILAYERS

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Fluorescence decay of DPH, TMA-DPH and DPH-PC in phosphatidylcholine (PC) vesicles, with varying unsaturation, was investigated using continuous Lorentzian lifetime distributions. Results showed that, with increasing temperature, lifetimes decreased for all three probes. However, due to the attachment of DPH to the sn-2 position of PC, the DPH-PC lifetime was less sensitive to the temperature change since its ability to sample across the lipid bilayer was restricted. Also, no distributional width (w) was recovered, indicating environmental homogeneity for DPH-PC. Results can be interpreted by considering the effect of dielectric constant gradient. In addition, w values tended to increase with decreasing temperature of the system, suggesting that the vertical sampling rate a dominating factor controlling the recovery of a fluorescence lifetime distribution.

W-Pos263

Deviation from Homeoviscous Adaptation In *Escherichia Coli* Membranes

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The process by which an organism changes the composition of its membranal fatty acids in response to growth temperature, so as to maintain optimal membrane functioning, is known as Homeoviscous Adaptation (HA). One expression of HA is the constancy of the fluorescence polarization (P) of the lipophilic probe 1,6-diphenyl-1,3,5-hexatriene (DPH) in membranes of cells grown at various temperatures. The P of DPH in the membranes of *E. coli* was shown by us to be inversely proportional to bacterial growth rate on different carbon sources. This result, implying failure of HA, is now complemented by measurements of DPH lifetimes, which indicate that the dominant variables contributing to the drop in P are (1) the order parameter of the membrane, which goes down and (2) the fluidity, which may slightly increase. These are then the changes induced by enhanced growth rate. Two additional effects, cell membrane permeability and sensitivity to thermal shock, determined by the diffusion of o-nitrophenylgalactoside (ONPG) and by exposure to 52°C, respectively, are reported to increase with growth rate. We can now conclude that there is a deviation from the principle of HA in *E.coli* grown at various rates, brought about by controlling the growth media at constant temperatures.

W-Pos264

EFFECT OF PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE ON MEMBRANE MICROHETEROGENEITY

MARY LOU WRATTEN

Phospholipid hydroperoxide glutathione peroxidase (PHGP) has previously been shown to reduce phospholipid hydroperoxides (PLOOH) to corresponding phospholipid hydroxides (PLOH). Cross correlation multifrequency phase fluorometry was used to determine DPH lifetime distributions in small unilamellar vesicles consisting of PL, PLOH, PLOOH or PLOOH with PHGP. All vesicles showed a bi-exponential decay and a decrease in both lifetime and distribution width as a function of temperature from 5 to 35°C. Addition of PHGP to PLOOH vesicles caused a 25% reduction in the fractional intensity of the long lifetime component and a 2-5-fold increase in the distribution width of the short lifetime component. These findings suggest that a consequence of PHGP-catalyzed reduction of PLOOH is to impart greater membrane microheterogeneity afforded by PLOH.

W-Pos265

SELECTIVITY OF INTERACTION OF CHOLESTEROL WITH GLYCERO-PHOSPHOLIPIDS AND SPHINGOMYELIN.

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Cholesterol is known to exhibit a strong immobilizing effect on lipids when the bilayer is in the liquid crystalline state. More interestingly, it has been suggested that cholesterol induces the formation of two liquid crystalline phases, namely l_o (liquid-ordered) and l_d (liquid-disordered). This effect of cholesterol on lipid dynamics is likely to be related to its spontaneous interbilayer exchange. Using spin-label ESR spectroscopy, we have measured the effect of cholesterol on six glycerophospholipids and three sphingomyelins of varying acyl chain composition and charge in the l_o and l_d phases. In the l_d phase, cholesterol affects the chain mobilities of all lipids to an equal extent. In the l_o phase, the interaction of cholesterol with sphingomyelin is the strongest. Increasing acyl chain unsaturation and headgroup charge lead to a stronger cholesterol-lipid interaction.

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W-Pos266

PHOTOBLEACHING KINETICS OF NBD-PE AND DiIC$_{18}$ in DOPC MULTIBILAYER MEMBRANES. L. Nakhimovsky, D. Chester, R. Hechtman and D.E. Koppel, Department of Biochemistry, UCONN Health Center, Farmington, CT 06032.

Irreversible, single-exponential decay of the fluorophore is a primary assumption to the FRAP technique employed for diffusion coefficient measurements. To evaluate the validity of this assumption for partially dehydrated multibilayer systems the concentration, hydration, and irradiation intensity dependence of photobleaching modes and their decay rates were studied. In contrast to NBD-PE, the photobleaching kinetics of DiIC$_{18}$ is strongly concentration dependent. At high DiIC$_{18}$ concentrations (above 10^{-4} mole/mole) three processes were distinguished while at low concentrations, only one process with a very small decay constant (4×10^{-8} $m^2/s\cdot W$) appeared. Decay constants for both lipid analogues dramatically decrease with increasing bilayer hydration becoming constant above 50% r.h. (4×10^{-8} and 4×10^{-7} $m^2/s\cdot W$ for DiIC$_{18}$ and NBD-PE respectively). The photobleaching process of NBD-PE and DiIC$_{18}$ (the latter only at low concentrations) in DOPC multibilayer membranes was predominantly single-exponential but high irradiation intensity levels elicited a second, faster mode of decay indicative of a possible two-photon process.

W-Pos267

CORRECTING FOR DYNAMIC BROADENING IN FLUORESCENCE PHOTOBLEACHING EXPERIMENTS-M. Bertch and D.E. Koppel, University of Connecticut Health Center, Farmington, CT 06032

In fluorescence photobleaching experiments with a focused laser spot, the calculated lateral diffusion coefficients (D) may underestimate the true values if the bleaching time is not sufficiently short in comparison to the characteristic diffusion time of the probe. From previous computer simulated bleaching experiments (M. Bertch and D.E. Koppel, 1988, Biophys. J. 53:512a), it was found that $\Gamma_d=(w(0)^2/w_o^2)$ as a function of parameters normally measured to calculate D, could be expressed as the product of two separable, empirical functions representing dynamic (change in the width of the fluorescence profile due to diffusion during the bleaching pulse) and static (bleaching in the wings of the laser profile) broadening. Γ_d has now been characterized experimentally in an artificial membrane system. The experimental data fit very well to the theoretical expression for Γ_d. With these results, more accurate values of D can now be determined from routinely measured experimental parameters, correcting for the effect of diffusion during the bleaching pulse. This work is supported by grant GM 23585 from the National Institutes of Health.

W-Pos268

A MULTIFREQUENCY PHASE FLUORESCENCE STUDY OF ANTHRACYCLINE ASSOCIATIONS WITH PHOSPHOLIPID BILAYERS: EFFECT OF ACYL CHAIN ORDER. T.G. Burke, H. Malak*, and J.H. Doroshow, Dept. of Medical Oncology, City of Hope National Medical Center, Duarte, CA 91010 and *Center for Fluorescence Spectroscopy, University of Maryland School of Medicine, Baltimore, MD 21201

We used frequency-domain fluorometry to determine the intensity and anisotropy decay kinetics of anthracycline anticancer drugs bound to small unilamellar vesicles of either dimyristoyl phosphatidylcholine (DMPC) or distearoyl phosphatidylcholine (DSPC) in PBS buffer at 37°C. Nine different anthracycline derivatives were studied; exciting light of 514 nm was modulated at multiple frequencies from 2 MHz to 1 GHz. Intensity decays for bound drugs appeared monoexponential (e.g. Adriamycin (ADM) in DMPC, τ=1.26 ns, α=1, f=1, X^2_R=1.3). Anisotropy decays for bound drugs were biexponential (ADM in DMPC, θ_1=0.40 ns, g_1=0.37, θ_2=∞, g_2=0.63, X^2_R=2.9; ADM in DSPC, θ_1=0.21 ns, g_1=0.35, θ_2=∞, g_2=0.65, X^2_R=2.9). Our results suggest that local anthracycline rotational motions were much more rapid in solid DSPC bilayers than fluid DMPC bilayers, suggestive of reduced drug penetration into the solid membranes.

W-Pos269

PYRENE EXIMER MAPPING IN CULTURED FIBROBLASTS BY RATIO IMAGING AND TIME-RESOLVED MICROSCOPY. James A. Dix & A.S. Verkman. CVRI, UCSF, CA.

The kinetics of pyrene eximer formation provides a measure of lateral diffusibility in bilayer membranes. Swiss 3T3 fibroblasts were labeled with pyrene, pyrene-decanoic acid (PDA) and 1,3-dipyrenylpropane (DPP) by incubation in the presence of Pluronic F127. Single cell emission spectra obtained by epifluorescence microscopy (excitation 350 nm) with photodiode array detection showed monomer (380-420 nm) and eximer (475 nm) peaks. The eximer-to-monomer fluorescence ratio (E/M) increased with increasing temperature and loading time. Time-resolved studies of fibroblasts labeled with PDA gave monomer and eximer lifetimes of 101 ns and 78 ns, with a monomer-to-eximer conversion rate of 0.02 ns^{-1}. E/M ratio images were obtained at 350 nm excitation and 405 ± 10 nm (monomer) and >470 nm (eximer) emission wavelengths. E/M ratios of PDA showed spatial variation across the cell with highest ratios at the peripheral plasma membrane. These results establish the methodology to label cells with eximer-forming probes and to image eximer distributions in membranes of intact cultured cells. E/M ratios are sensitive to maneuvers which alter the membrane physical state and should be of utility in examining the cellular regulation of membrane fluidity.

W-Pos270

VASOPRESSIN DOES NOT ALTER FLUIDITY OF COLLECTING TUBULE MEASURED BY ORIENTATION-INDEPENDENT ANISOTROPY IMAGING AND PICOSECOND MICROFLUORIMETRY. Kiyohide Fushimi and A.S. Verkman, CVRI, UCSF, CA.

Indirect evidence suggests that action of VP is the increase in apical membrane fluidity and/or cytoplasmic viscosity of target cells. To measure apical fluidity, cortical collecting tubules (CCT) from rabbit were perfused with TMA-DPH. Anisotropy (r) was mapped by recording images of the stained tubule with an emission polarizer parallel and perpendicular to 7 excitation polarization directions (Fushimi et al, Biophys J, in press). Lifetimes were measured by time-correlated photon detection. To measure cytoplasmic viscosity, r of tubules labeled with BCECF was imaged (Dix and Verkman, Biophys J, in press) and rotational correlation time (t_c) was measured by phase modulation. Water permeability (P_f) measured in real-time (Kuwahara et al, Biophys J 54:595, 1988) increased 15 to 260 x 10^{-4}cm/s with serosal VP (250uU/ml). TMA-DPH r in CCT apical membrane -VP, 0.257 ± 0.003 (n=7, SE), did not differ from r +VP, 0.258 ± 0.004; TMA-DPH lifetime (5.8±0.4ns) also did not differ. BCECF r and t_c -VP, 0.11±0.01 and 390±20, did not differ from values +VP, 0.11±0.01 and 380±10. We conclude that VP does not alter the fluidity of target epithelial cells.

W-Pos271

RESOLUTION OF LIPID CONFORMATIONS IN BIOLOGICAL MEMBRANES BY FREQUENCY-DOMAIN FLUORESCENCE SPECTROSCOPY. T.C. Squier, W. Wiczk, M. Fishman, and J.R. Lakowicz. Univ. of Maryland at Baltimore.

We have utilized frequency-domain fluorescence spectroscopy to directly recover the time-dependent terms related to the distribution of conformations of a fatty acid analog containing two chromophoric groups that function as a resonance energy transfer couple, i.e., 16-(9-anthroyloxy)-palmitoyl tryptophan methyl ester. In isotropic solvents the conformational distribution is best fit by a broad Gaussian distribution of distances, in agreement with simulations using an isomeric state model. Upon incorporation into single component model membranes containing either DMPC or DOPC the conformational distribution is best fit by a narrow Lorentzian distribution both above and below the phase transition temperature. The separation between the probes indicates that the anthroyloxy probe is located near the center of the bilayer, while the narrow distribution in the separation between the two chromophores emphasizes both the extended conformation of all the fatty acyl chains, as well as the specificity of the anthroyloxy probe to measure the dynamics at defined depths relative to the membrane normal.

W-Pos272

EFFECTS OF CHOLESTEROL ON THE ANISOTROPIC ROTATIONAL MOTIONS OF PERYLENE: A FLUORESCENCE PROBE FOR STUDYING LIPID PACKING. Lesley Davenport[#] and Yi Ri[*], Dept. of Chem., Brooklyn College of CUNY, Brooklyn, N.Y. 11210.

We have used perylene, an apolar fluorescence probe, for studying the effects of cholesterol on phospholipid packing within dimyristoylphosphatidylcholine (DMPC) small unilamellar vesicles (SUVs). The shape of perylene can be approximated by a disc exhibiting highly asymmetric in-plane and out-of-plane rotational movements. We have studied these anisotropic motions in lipid bilayers in the presence and absence of cholesterol by selective excitation of either the negative (256nm) or positive (410nm) absorption bands, which for perylene lie in the plane of the molecule and at right angles to each other. Emission anisotropy (<r>) values measured for perylene labelled DMPC SUVs in the absence of cholesterol, at various excitation wavelengths, reflect both hindered in-plane and out-of-plane rotations. In aggrement with earlier studies (Kowalczyk *et al.*, Fourth Conference in Luminescence (1982) 187), the in-plane rotational rate increases with increasing temperature and appears sensitive to the physical state of the lipid membrane. In contrast, out-of-plane probe motions appear invariant to temperatures below the main lipid phase transition. With addition of cholesterol (20mole%), the in-plane rotational rate of perylene is severely restricted. Out-of-plane rotations are apparently not affected. Interpretations of our data in terms of a heterogeneous compartmental lipid packing model will be discussed. (Supported in part by the [#]Petroleum Res. Fund, a [#]PSC-CUNY award, and by [*]GM08078-05 MARC Program).

W-Pos273

INTERACTIONS OF LAURDAN AND PRODAN WITH MEMBRANES AT HIGH PRESSURES

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The fluorescence emission spectra of Laurdan (2-dimethylamino-6-lauroyl-naphthalene) and Prodan (6-propionyl-2-(dimethylamino)naphthalene) in lipid vesicles were measured as a function of pressure (0.001 - 3 kbar). No abrupt changes in F435/F510, the ratio of fluorescence intensity at 435 nm to that at 510 nm, are seen for Prodan in EggPC (MLV) at 19°C. In contrast, the F435/F510 of Laurdan in EggPC (MLV) shows an abrupt increase at about 2.0 kbar at 19°C. This result suggests that a pressure-induced ordering takes place in the hydrocarbon side chain of Laurdan, which stabilizes the "less polar" disposition of the chromophore and shifts the equilibrium away from the "polar" disposition (Chong (1988) Biochemistry 27, 399). The fluorescence data also suggests that, like Prodan, Laurdan is not squeezed out of the membrane by pressure in the pressure range examined. (Supported by the ARO, NSF-MRCE, and AHA-EI).

W-Pos274

CATION-INDUCED PHASE SEPARATION IN PHOSPHATIDIC ACID VESICLES MONITORED BY CATION-CATION ENERGY TRANSFER. Matthew Petersheim & Joanne Sun, Chemistry Dept., Seton Hall Univ., South Orange, NJ 07079.

Fluorescence energy transfer between the lanthanides, Ce(III) and Tb(III), was used to monitor cation-induced phase separation in phosphatidic acid vesicles. The kinetics of phase separation depends on temperature, pH and lanthanide surface concentration, all of which will be shown. There are distinct differences in the process for dimyristoylphosphatidic acid and dioleoylphosphatidic acid. The latter is known to form inverted micelles or the H_{II} phase in the presence of Ca(II); this may be responsible for the observed differences. Nucleation of the Ce(III)/Tb(III) centers occurs on a subsecond timescale while extensive phase separation is very much slower and may require critical surface concentrations.

The very strong absorption bands of Ce(III) and efficient transfer of energy to Tb(III) promise a very useful probe of millisecond events at nanomolar concentrations.

W-Pos275

DIPYRENYL-PHOSPHATIDYLCHOLINE PROBES: THERMODYNAMICS OF INTRAMOLECULAR EXCIMER FORMATION IN MODEL MEMBRANES.

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We have measured the pressure dependence of the intramolecular excimer formation rate, K, of dipyrenylphosphatidylcholine (dipyPC) probes in model lipid multilayer vesicles as a function of temperature. The analysis made use of the equation $K=(r/r^*)\tau_m^{-1}$, (Biophys.J., in press) where r is the temperature and pressure dependent excimer/monomer intensity ratio of dipyPC, τ_m is the lifetime of the isolated monopyrenyl analog (pyPC) and r* is proportional to the ratio of excimer to monomer lifetimes and was obtained by fitting the probe-ratio dependent pyPC monomer and excimer yields according to the milling crowd model (Biophys.J. 1986, 49:987). The temperature dependence of the pressure-induced phase transition agrees well with that obtained by other techniques, confirming that dipyPC accurately reports changes in membrane fluidity. The volume of activation of K in various model lipid multilayers in the liquid-crystalline phase was found to decrease with increasing temperature, supporting our previous conclusions (Biophys.J., in press) that the intramolecular excimer formation process in dipyPC is rate-limited by the availability of free volume within the surrounding lipid matrix.
Supported by N.I.H. GM39924 and R24-RR05272.

W-Pos276

PARTICLE COUNTING AND DIFFUSION MEASUREMENTS IN NONIDEAL SOLUTIONS BY FLUORESCENCE CORRELATION SPECTROSCOPY. James R. Abney (1,2), Bethe A. Scalettar (1,2) & Charles R. Hackenbrock (2). (1) Life Sciences, Lawrence Berkeley Laboratory, Berkeley, CA 94720; and (2) Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Here we show how interparticle interactions affect the initial amplitude, g(0), of the FCS autocorrelation function. The results are applied to the study of particle counting, aggregation, and interaction-dependent diffusion. In ideal solutions, $g(0) = 1/\langle N \rangle$, where $\langle N \rangle$ is the average number of particles in the observation volume. In nonideal solutions, the generalized expression is $g(0) = kT\kappa/V$, where k is Boltzmann's constant, T is the temperature, κ is the compressibility, and V is the observation volume. Since κ is generally decreased by repulsions and increased by attractions, use of ideal FCS relationships could lead to an overestimate of particle number in repulsive systems and an underestimate in attractive systems. Order-of-magnitude errors may result when ideal relationships are applied to biological membranes. Noting that κ is inversely proportional to the mutual-diffusion coefficient, we show how g(0) may be used to isolate the effects of direct interactions on mutual diffusion and thus can be used to assess the validity of theories of interaction-dependent diffusion.

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W-Pos278

MAGNETIZATION EXCHANGE BETWEEN H_2O AND BIOLOGICAL LIPID BILAYERS

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We have observed magnetization exchange between H_2O and tissues containing high lipid content, such as kidney and brain. The magnetization exchange between bulk water and the immobile component can be measured by saturating the macromolecular matrix with traditional magnetization transfer techniques. This approach can be used to map the interaction of free water with different types of lipid bilayers. Our experiments demonstrate that bilayers do contribute to magnetization exchange in biological tissues. In addition, cholesterol greatly enhances this process. We observed magnetization exchange rate constants of 0.06 sec^{-1} to 1.1 sec^{-1} between water and lecithin bilayers containing 0 to 80 mole % of cholesterol. It is not clear if this results from a change in lipid correlation time or number of waters associated with the lipid. In addition, the exchange process was sensitive to the amount of lipid present, with abolition of the effect below 30 wt % of lipid.

W-Pos277

NEW LONG WAVELENGTH FLUORESCENT MEMBRANE PROBES. Hee Chol Kang and Richard P. Haugland, Molecular Probes, Inc., Eugene OR 97402.

Fluorescent lipid analogs have been used extensively to measure membrane properties. With the exception of the nitrobenzoxadiazole (NBD) fluorophore, almost all of the long wavelength fluorophores that can be excited by the argon laser at 488 and 514 nm have electronic charges that limit their access to the lipid portion of the membrane. We have synthesized and spectrally characterized several new fluorescent membrane probes derived from the new Bodipy™ fluorophore and a Nile Red-like oxazine fluorophore.

The Bodipy fluorophore has absorbance and fluorescence properties similar to fluorescein but is sufficiently lipophilic that it remains in the membrane. Its quantum yield is relatively unaffected by the environment. In contrast, the oxazine fluorophore is almost non-fluorescent in water or polar solvents but is very fluorescent in lipids. Typically the spectra of the oxazines show maximum absorbance and emission near 555 and 630 nm in MeOH and 545 and 590 nm in chloroform. Fatty acid, phospholipid, coenzyme A, cholesteryl ester, ceramide, sphingomyelin, psychosine, glucosamine, phorbol, diacylglycerol and other derivatives have been prepared from these two fluorophores. Supported by NIH grant 1 R44 GM 37347.

W-Pos279

SECOND ORDER EFFECTS IN THE INTERACTION OF ELECTRIC FIELDS WITH CELL MEMBRANES. B. Ehrenberg, M. Wei, & L.M. Loew, Dept. of Physiology, U. Conn. Health Center, Farmington, CT 06032.

An external electric field induces a change in membrane potential which integrates to zero over a closed surface, as given by the solution to Laplace's equation for a given cell geometry. In this paper we report the effects of long term exposure of individual HeLa cells to medium electric fields (10-70 mV/cell diameter). We measure both the resting membrane potential and the spatially resolved membrane potentials induced by probing electric field pulses. These new measurements of resting potential and long term effects are made possible by 2 technical advances: a new dye, di-8-ANEPPS, which solves the problem of slow dye internalization; a dual wavelength ratiometric fluorescence imaging approach which solves the problem of uneven staining. The results indicate that membrane potential is attenuated by long term exposure to external electric fields. (Supported by USPHS grant GM35063).

W-Pos280

INTER- AND INTRAMOLECULAR EXCIMER FORMATION OF PYRENE-LABELED LIPIDS IN LAMELLAR (L) AND INVERTED HEXAGONAL (H) PHASES

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The lateral mobility and geometrical packing of lipids in lamellar (L) and inverted hexagonal (H) phases were studied with mono- and dipyrenyl phosphatidylcholine (PC) probes, respectively. The rates of excimer formation of the probes in binary mixtures of phosphatidylethanolamine (PE)/ diacylglycerol and PE/PC were determined from steady-state fluorescence spectra and fluorescence lifetimes at various lipid compositions and temperatures. The excimer formation rate of dipyrenyl PC decreased while that of monopyrenyl PC increased at the L to H phase transition by varying the lipid composition. An opposite trend was observed at this phase transition by increasing the temperature. For the dipyrenyl PC, it is concluded that the intramolecular collisional rate of the two pyrene moieties depends both on the dynamics and geometry (spraying) of the lipid acyl chains. For the monopyrenyl PC, the intermolecular collisional rate depends on the dynamics and free volume of the lipid hydrocarbon region.

W-Pos281

INFRARED AND FLUORESCENCE SPECTROSCOPIC STUDIES OF LIPID NON-BILAYER PHASES

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Fourier Transform Infrared (FTIR) and Fluorescence spectroscopy was used to investigate the polymorphic phase behavior of binary lipid mixtures containing unsaturated phosphatidylethanolamine (PE). The lipid vibrational bands due to CH_2 stretching, CH_2 bending and C=O stretching modes at $\sim$ 2850, 1460 and 1735cm^{-1}, respectively, were measured at different lipid compositions and temperatures. For the diolein/egg PE mixture, the lamellar gel (L_b) to lamellar liquid crystalline (L_a) and the L_a to inverted hexagonal (H_{II}) transitions were observed at $\sim$ 15°C and 55°C, respectively, for 0% diolein. As the diolein content increased from 0 to 12%, the L_a-H_{II} transition temperature decreased drastically while the L_b-L_a transition temperature decreased slightly. At 12% diolein, a direct transition from the L_b to H_{II} phase was found at $\sim$10°C. Similar measurements were also performed on a PC/PE mixture which exhibits other metastable phases, such as isotropic, amorphous and cubic phases. The above FTIR results were then compared with the rotational and hydration dynamics results obtained from time-resolved fluorescence measurements on the same lipid systems.

W-Pos282

EFFECT OF LIPID COMPOSITION ON SOLUBILITY AND ORDERING OF n-ALKANES AND n-ALKANOLS IN BILAYER MEMBRANES.

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The solubility of n-alkanes, and the ordering at the 1-CH_2 segment of n-alkanols in model bilayer membranes, has been determined as a function of lipid composition by ^2H NMR. For bilayers similar in composition to synaptosomal membranes, a break in n-alkane solubility at n-nonane is observed. For n-alkanols in the same model systems, a maximum in ordering is found at n-dodecanol, without any change in solubility. These breaks in physical parameters with changing solute chain length correspond to the cut-off points in anesthetic potency in each of these homologous series. (Supported by an Academic Challenge Grant from the Ohio Board of Regents.)

W-Pos283

FLUORESCENCE MEASUREMENTS OF ACTIVATED HUMAN NEUTROPHILS DURING THE RESPIRATORY BURST PHENOMENON.

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The respiratory burst of human polymorphonuclear leukocytes is triggered by a number of soluble agents. The interaction between the soluble stimulus and the cell surface induce a number of molecular and functional modifications of the plasma membrane.

Using non-physiological and physiological activators of the respiratory burst we studied steady-state fluorescence anisotropy and excited state decay of 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene(TMA-DPH) in intact human polymorphonuclear leukocytes. TMA-DPH fluorescence decay was measured by multifrequency phase fluorometry and the data were analyzed using a continuous distribution of lifetime values. The respiratory burst phenomenon has been shown to take place concomitantly with an increase of steady-state fluorescence anisotropy and with changes of fluorescence lifetime values and distributions. This approach enables us to study changes of membrane heterogeneity in intact cells.

Supported by a grant from CNR 88.00482.04 G.C.

W-Pos284

E. COLI PHOSPHATIDYLETHANOLAMINE NEAREST NEIGHBORS CAN BE DETERMINED BY CHEMICAL CROSS-LINKING. Mary R. Roth and Ruth Welti, Division of Biology, Kansas State University, Manhattan, KS 66506.

The phospholipids of *Escherichia coli* include approximately 80% phosphatidylethanolamine species. Thus, most of the diversity in the phospholipid molecular species in these cells resides in the acyl chains of phosphatidylethanolamine. Phospholipids derived from *E. coli* strain AB1623 were treated with the amine-specific cross-linking reagent, dimethylsuberimidate. The dimeric phosphatidylethanolamines were separated by reverse phase HPLC, derivatized to acetyldiacylglycerols, and analyzed by argentation TLC and GLC. In this mixture of fluid-phase lipids, the phosphatidylethanolamine species were, as a first approximation, randomly arranged. (Supported by NIH grant GM 36797 to RW).

W-Pos285

EFFECTS OF ETYA ON WHOLE CELL MEMBRANE FLUIDITY IN U937 and PC-3 CELLS. BROWN, M.D. and Anderson, K.M.

5,8,11,14 eicosatetraynoic acid (ETYA) is an arachidonic acid (AA) analogue which acts as both a competitive inhibitor and agonist of AA. ETYA inhibits DNA synthesis in both U937 and PC-3 cell lines. The signal pathways responsible are not yet known however, changes in the physical property of the cell membrane, specifically fluidity, may play a role.

Whole cell membrane fluidity in U937 and PC-3 cells was measured using steady-state fluorescence polarization with the probe 1-[4-(trimethylamino)phenyl]-6-phenyl hexa-1,3,5-triene (TMA-DPH) at 60 seconds after incubation with DMSO (control), AA or ETYA. Fluidity was described as the order parameter (s) which is inversely related to the membrane fluidity.

Order parameter values for control, AA and ETYA were 1.22 ± .03, 1.09 1 .06 and .97 ± .05 respectively in PC3 cells. These represent a 16.4% and 8.5% increase in membrane fluidity in U937 and PC-3 cells respectively, ETYA changes the physical property of the cell membrane, specifically fluidity, which in turn may play a role in the inhibition of DNA synthesis evoked by ETYA.

W-Pos286

AMINO ACID PERMEABILITY ACROSS LIPOSOME BILAYERS. (E.A. Harang and D.W. Deamer, Department of Zoology, University of California at Davis, Davis, CA 95616.)
We studied amino acid permeability across PC:PG liposome bilayers. Aliquots of PC:PG (9:1 molar ratio) liposomes containing 1mM amino acid at pH 9 were analyzed at various times by a fluorometric assay to determine amino acid leakage from the liposomes over time. The permeabilities are on the order of 10^{-8} to 10^{-9}. The order of permeability at pH 9.0 is glutamate, arginine, valine, isoleucine, leucine, methionine, phenylalanine and tyrosine. Glutamine, cysteine, aspartate and glycine are less permeable than tyrosine and have equivalent permeabilities. Alanine was the least permeable. The relative permeability of a given amino acid appears to rely on the character of the R groups; those groups which are more hydrophobic tend to confer higher permeability on the amino acid. These general results correlate with previous studies of amino acid permeability coefficients, hydrophilicity, hydrophobicity values.

W-Pos287

Ca^{2+} UPTAKE BY LIPOSOMES MODELED ON THE INNER MITOCHONDRIAL MEMBRANE: CATION SELECTIVITY AND DEPENDENCE ON LIPOSOME CONCENTRATION. Rena G. Lapidus & Patricia M. Sokolove, Dept. Pharmacol. & Exp. Ther., Univ. Maryland Med. School, Baltimore, MD.

Liposomes composed of phosphatidylcholine (PC)/phosphatidylethanolamine (PE)/ and cardiolipin (CL) accumulate Ca^{2+}. Uptake is biphasic, but is dominated by the slow, highly temperature-dependent second phase [Kester & Sokolove (1989) Biochim. Biophys. Acta 980: 127]. Cation uptake into PC/PE/CL liposomes prepared by extrusion and loaded with either Arsenazo III or Antipyrylazo III was further characterized with the following results: (1) The slow transport process was selective for Ca^{2+} over Sr^{2+}, Ba^{2+}, and Cd^{2+}. (2) Decreasing the liposome concentration used in uptake measurements suppressed the initial rapid uptake component and enhanced the more prominent, slower phase. The latter observation suggests that the bulk of Ca^{2+} transport in this system is mediated by intrabilayer lipidic particles. Rate calculations based on the work of Siegel [(1984) Biophys. J. 45:399] are consistent with this alternative. [Supported by NIH (HL32615) and the Graduate School, Univ. MD, Baltimore]

W-Pos288

MODULATION OF THE ION SELECTIVITY OF COLICIN E1 CHANNELS BY pH AND BILAYER COMPOSITION. James O. Bullock and Mary R. Reilly. *Department of Physiology, University of Missouri-Columbia, Columbia, MO 65212*

Colicin E1 is a bacterial toxin which forms voltage dependent channels in planar lipid bilayers. The selectivity of this channel is known to be influenced by the pH on both sides of the membrane. In order to eliminate the effects of a negative surface charge, we have studied the Na^+ *vs.* Cl^- selectivity of this channel in the neutral lipids diphytanoyl lecithin (DPhPC) and bacterial phosphatidyl ethanolamine (BacPE). In membranes composed of DPhPC, colicin E1 was highly selective for Cl^- when the pH of the *cis* compartment was 4.0. The selectivity was independent of the *trans* pH, and was refractory to increases in the *cis* pH. When the initial *cis* pH was 5.0 or greater, increasing the pH on either side of the membrane increased the cation selectivity of the channels and *vice versa*. All membrane associated channels, whether opened or closed, were refractory at *cis* pH 4.0, but aqueous protein remained responsive. In membranes composed of BacPE, the dependence of selectivity on pH was similar to that in DPhPC, but no evidence of refractory behavior was observed. These results suggest that low pH decreases the cation permeability of colicin E1 *via* the influence of titratable residues upon the global conformational state of the protein, rather than by protonation of a cation binding site within the channel lumen. (Supported by GM37396)

W-Pos289

THE VOLTAGE-DEPENDENT ACTIVITY OF ALAMETHICIN IN SMALL UNILAMELLAR VESICLES S. J. Archer and D. S. Cafiso Department of Chemistry, University of Virginia, Charlottesville, VA 22901.

Several models have been proposed to explain the voltage-dependent ion transport activity of alamethicin in planar bilayers. Some of these models are based on a voltage-dependent conformational change of the membrane bound peptide (for example, see G. Boheim *et al.*, 1983, Biophys. Struct. Mech. 9:181; J. E. Hall *et al.*,1984, Biophys. J. 45:233), while other models are based on voltage-dependent partitioning of the peptide into the lipid bilayer (V. Rizzo, *et al.*, 1987, Biochemistry 26:2751). In an effort to separate partitioning effects from other factors, we studied the current-voltage behavior and partitioning of alamethicin in small unilamellar vesicles. The partitioning of the peptide into the lipid bilayer was investigated using a spin-labeled derivative of alamethicin. The current-voltage behavior was examined using a combination of potential-dependent spin probes. Under conditions where all of the peptide is membrane bound, alamethicin was found to have a voltage-dependent activity. Although these results do not rule out some role for voltage-dependent partitioning effects, they do indicate that a potential-dependent change in the conformation or in the aggregation of lipid-associated peptide is occurring.

W-Pos290

EFFECTS OF MICROWAVE FIELDS ON LIPOSOME PERMEABILITY: NON-PHASE TRANSITION LIPOSOME VESICLES. R.P. Liburdy and B. Fingado, Lawrence Berkeley Laboratory, Bioelectromagnetics Research Facility, UC Berkeley, Berkeley, CA 94720

Microwave fields have been shown by us to influence the permeability of phase-transition liposome vesicles (Radiation Research 108; 102-111:1986). A marked increase in drug permeability occurs at temperatures below the phase transition temperature, Tc. This microwave effect (2450 MHz, SAR 6-60 mw/gm) is potentiated by plasma and by oxygen and is inhibited by antioxidants. We have also observed that the dielectric properties of these vesicles are altered at the phase transition temperature (Phys. Med. Biol. 33; 1309-1324:1988). Recently we have performed experiments in which non-Tc liposomes were treated with microwaves at 37°C. Large unilammellar and multilamellar vesicles were formed from PI:PC and from partially hydrogenated forms of these phospholipids; 6-carboxyfluorescein or doxorubicin was employed as a drug release marker. We observed an approximate 50-55% maximal release when these liposomes were treated with microwaves compared to 10% in the absence of the field. Plasma enhanced this differential permeability. The presence of antibody in the bilayer did not have a significant effect on this microwave release. Work supported in part by the US Department of Energy under contract DE-AC-03-76SF000098.

W-Pos291

DIFFRACTION STUDIES ON A GRAMICIDIN/CESIUM THIOCYANATE COMPLEX. K. Ravikumar and B. A. Wallace, Department of Chemistry and Center for Biophysics, Rensselaer Polytechnic Institute, Troy, New York 12180.

The linear pentadecapeptide gramicidin A offers an excellent model compound for the selective transport of ions across lipid bilayer membranes. In continuation of our studies on the interactions of ions in the gramicidin pore, crystals of a gramicidin/cesium thiocyanate complex were prepared. Crystals grown at room temperature by slow evaporation belong to the space group $P2_12_12_1$ with a=31.886, b=52.560, and c=31.249 Å. Diffraction data were collected to 1.8 Å on a Nicolet/Xentronics area detector (Ravikumar, Muchmore, Einspahr, and Wallace, ACA Abstracts, 17:116,1989). Patterson calculations revealed that there are four cesium sites in each unit cell and that these sites are at different positions when compared with the cesium sites in crystals of a gramicidin/cesium chloride complex (Wallace and Ravikumar, Science, 241: 182,1988). The data has been phased using the combined or choice phases derived from the anomalous scattering and the partial structures of the cesiums. An initial electron density map has been calculated.

W-Pos292

THE PROTEIN-LIPID STRUCTURE OF STRATUM CORNEUM IN RELATION TO ITS PHASE AND PERMEABILITY PROPERTIES: **M. Francoeur, J. Humm & R. Potts, Pfizer, Groton, CT, and B.Ongpipattanakul & R. Burnette, Univ. of Wis., Madison, WI.** The outer layer of mammalian epidermis or stratum corneum(SC) consists of keratinized cells dispersed in a lipid matrix. These lipids represent ~ 15% of the total SC weight consisting of ceramides(45%), cholesterol(25%) & fatty acids(15%). This unique combination of lipids are arranged extracellularly as bilayers and have been postulated to constitute the main permeability barrier for skin. Here, we have characterized the structural conformations for the protein & lipid elements of the SC using FT-IR and DSC. With vapor phase diffusion studies, the P^H_{2O} has been found to depend on the lipid, but <u>not</u> the protein structure of the SC. It seems that the transport of H_2O across the bilayer and the formation of gauche conformers along the lipid alkyl chain have the same functional dependence on temperature. This finding suggests that the diffusion of H_2O across the skin is limited by the micro-environment of the SC lipids. Incorporation of oleic acid(OA) into the bilayer increased the transport of H_2O and reduced Ea from 14 to 11 kcal/mol. Eyring analysis indicated that the OA reduced both $\Delta S^{\#}$ and $\Delta H^{\#}$. FT-IR results with ^{2}H-OA demonstrated that microphase separation had occurred within the SC lipid bilayer. This chemically-induced phase separation is analogous to what has been hypothesized to explain increased permeability of phospholipid vesicles to Na^+ when at their Tm.

W-Pos293

METHOD OF ORIENTED CIRCULAR DICHROISM (OCD). Huey W. Huang, Yili Wu & Glenn A. Olah, Physics Department, Rice University, Houston, TX 77251

A new method for determining the orientation of α-helical sections of proteins or peptides in membrane is presented. To apply this method, membranes containing proteins must be prepared in a multilayer array. Circular dichroism spectra of the multilayer sample are then measured at the normal as well as oblique incident angles with respect to the bilayer planes; we call such spectra oriented circular dichroism (OCD). The procedure of OCD measurement, particularly the ways to avoid the spectral artifacts due to the effects of dielectric interfaces, linear dichroism and birefringence, and the method of data analysis are described. To illustrate the method, we analyze the OCD of alamethicin in diphytanoylphosphatidylcholine multilayers (DPhPC). We conclude unambiguously that the helical section of alamethicin is parallel to the membrane normal when the sample is in the full-hydration state, but the helical section rotates to the plane of membrane when the sample is in a low-hydration state. We also obtained the parallel and perpendicular CD spectra of α-helix, and found them to be in agreement with previous theoretical calculations based on the exciton theory. These spectra are useful for analyzing protein orientations in future experiments.

W-Pos294

The States of Alamethicin Interacting with Lecithin Bilayers

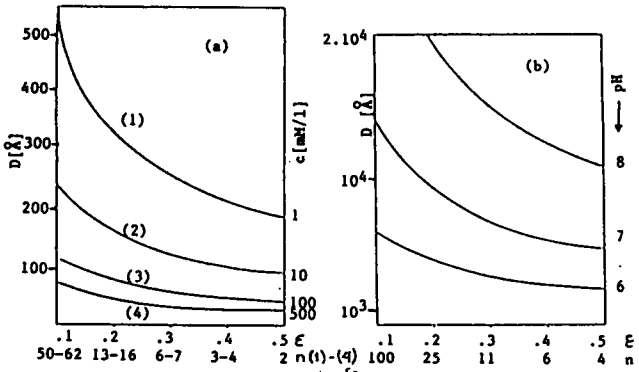
Yili Wu, Huey W. Huang & Glenn A. Olah, Physics Department, Rice University, Houston, TX 77251

To understand the molecular mechanism of the alamethicin channel, it is essential to understand how the peptide interacts with bilayer membranes. We used the method of oriented circular dichroism (OCD; see POSTER by Huang et al.) to study the orientation of alamethicin mixed in dilauroyl-, dimyristoyl-, dipalmitoyl-, diphytanoyl- and dioleoyl- phosphatidylcholine (DLPC, DMPC, DPPC, DPhPC & DOPC) multilayers as we varied the partial pressure of the equilibrating water vapor. In DPhPC and DOPC, the α-helical section of alamethicin is perpendicular to the plane of membrane when the samples are fully hydrated; however, it rotates to the plane of membrane in a low-hydration state (e.g., 50% r.h.). In DLPC and DMPC, the α-helical section is always perpendicular to the plane of membrane, but there is a slight conformation change in the secondary structure when the hydration is decreased from 100% r.h. The implication of these observations will be discussed.

W-Pos295

LOCAL TRANSIENT FLUCTUATIONAL DENSITY AS PRODUCING IONIC FLOW THROUGH CELL MEMBRANES. J.Procópio and J.A.Fornés. (Intro. by S.H.White)-(1) Departamento de Fisiologia e Farmacologia USP-Brazil.(2)Department of Physiology and Biophysics, University of California,Irvine.

We analize some hypothetical conditions in which random fluctuations of ion concentration in restricted regions might lead to transient ion flow through a cell or mitochondrial membrane, and to eventually produce ion concentrations gradients across these structures. We observe from figures that the larger the required relative fluctuation ε , the smaller is the corresponding volume enclosing it. However, there is a broad range of concentrations for which significant fluctuations of c take place in enclosing volumes having diameters comparable to the membrane thickness. In these cases, the dissipation of a fluctuation close to the membrane could result in a transient net flow of the corresponding ion through the channel. In the case of protons the fluctuation in concentration should have considerable significance, due to the very low basal H+ concentration. For example, in a volume of one cubic micrometer there are about 60 free protons at pH 7 and this number should fluctuate between 52 and 68, what should produce significant changes in the local pH.
(Partially supported by CNPq-Brazil grant 200463/89-6/BF).



FLUCTUATION SIZE (D) VERSUS ITS RELATIVE AMPLITUDE ($\varepsilon = \frac{\delta c}{c}$) AND NUMBER OF THE PARTICLES ENVOLVED (n) FOR TYPICAL VALUES OF CONCENTRATIONS (c) AND pH OF BIOLOGICAL SYSTEMS. (a) IONIC FLUCTUATION IN GENERAL (b) PROTONIC FLUCTUATIONS.

W-Pos296

THE APPARENT PERMEABILITY COEFFICIENT FOR PROTON FLUX THROUGH PHOSPHATIDYLCHOLINE VESICLES IS DEPENDENT ON THE DIRECTION OF FLUX

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A dioleoylphosphatidylcholine unilamellar vesicle model system was used to determine proton permeability. The fluorescence of the pH reporter group, pyranine, trapped within vesicles with a different pH across the bilayer, was digitized and analyzed using a novel model with numerical integration. The permeability coefficients calculated were within the range of previously published values (1-4 x 10^{-4} cm/sec) but they were found to vary with the direction and size of the pH change across the vesicle. When the exterior pH was more acid (and constant) than the interior, the permeability was found to vary with the pH difference and the interior pH as it approached 7.2 at equilibrum. When the exterior was more alkaline (and constant) than the interior, the permeability was constant with pH but still was a function of the size of the gradient as the pH approached 8.0 at equilibrum. This behavior could not be explained by insufficient valinomycin, or heterogeniety in the size or permeability of the vesicles. A carrier mechanism can describe the observed effects.
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