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Analysis of Hofmeister Effects on Protein Adsorption At Aqueous-Solid Interfaces

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The effects of various salts on the adsorption of ribonuclease A (RNase) at a hydrophobic poly(styrene) film was analyzed in this study applying neutron reflectometry. It has been found that both the kosmotropic salts, $(NH_4)_2SO_4$ and Na_2SO_4, and the chaotropic salts, NaSCN and $Ca(SCN)_2$, significantly reduce the amount of adsorbed protein. Maximum adsorption is observed in the presence of NaCl. Apparently, there is no single Hofmeister effect on the degree of protein adsorption at an aqueous-solid interface which ranges from kosmotropic to chaotropic ions. The observed variations in the adsorbed amount can be attributed to variations in the packing density of the adsorbed protein molecules. The results suggest that kosmotropic salts reduce the degree of protein adsorption by disfavoring a conformational adaptation and a dehydration of the protein molecules at a hydrophobic poly(styrene) film. On the other hand, chaotropic salts shield hydrophobic interactions between the protein molecules and the substrate by saturating hydrophobic patches on the protein surface. Remarkably, the results of this study corroborate earlier findings on the effects of non-ionic cosolvents, glycerol and urea, on protein adsorption.

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Characterization of PriB Protein From *klebsiella Pneumoniae*

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PriB is a primosomal protein required for PriA helicase-dependent DNA replication restart. *Escherichia coli* PriB protein (*Ec*PriB) exists as a homodimer, and each polypeptide has 104 residues. Significant variation is found in length of *priB* gene from different organisms. In *Klebsiella pneumoniae* (*Kp*), the *priB* gene consisted of 168 nucleotides encoding a gene product of 55 amino acid residues. Sequence alignment indicates that *Kp*PriB lacks an N-terminal region (aa 1-49) found in *Ec*PriB, in which several key residues have proved to be a major role for interactions with PriA helicase and DnaT. In the present study, the properties of single-stranded DNA (ssDNA) binding, self-association, and primosome assembly of *Kp*PriB were further investigated. Based on these results, the structure-function relationships of *Kp*PriB are discussed.

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Design of Novel Methods to Eliminate DNA Binding to Recombinant Proteins

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Successful recovery of pure recombinant proteins after expression and purification is a constant challenge in molecular biology. Along with the need for purity there is also a need for high yield recovery. We have determined another challenge to pure recombinant protein expression in that proteins with high isoelectric points can bind to host bacterial DNA. We have demonstrated the presence of the protein/DNA complex using fluorescence spectroscopy. The focus of this study is to develop expression and purification methods to eliminate host bacterial nucleotide contamination. Data will be presented, using proteins with varying pI values, namely, C2B, FGF-2 , and FGF-1 on the presence and elimination of this DNA/protein complex. Studies will be presented on the effect of pH, salt, DNAase, and phospholipase on eliminating contaminant bound DNA. The effect of the host bacterial contaminant bound DNA on the structure and function of recombinant proteins will be assessed using various biophysical techniques, including multidimensional NMR spectroscopy.

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Hydration Potential of Lysozyme: Protein Dehydration Using a Single Microparticle Technique

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For biological molecules in aqueous solution the hydration pressure as a function of distance from the molecular surface represents a very short-range repulsive pressure that limits atom-atom contact, opposing the attractive van der Waals pressure. Whereas the separation distance for molecules that easily arrange into ordered arrays (e.g., lipids, DNA, collagen fibers) can be determined from x-ray diffraction, many globular proteins are not as easily structured. Using a new micropipette technique, spherical, glassified protein microbeads can be made that allow determination of protein hydration as a function of the water activity (a_w) in a surrounding medium (decanol). By adjusting a_w of the dehydration medium, the final protein concentration of the solid microbead is controlled, and ranges from 700 to 1150 mg/ml. By controlling a_w (and thus the osmotic pressure) around lysozyme, the repulsive pressure was determined as a function of distance between each globular, ellipsoid protein. For separation distances, d, between 2.5 and 9 Å, the repulsive decay length was 1.7 Å and the pressure extrapolated to d 0 was 2.2 x 10^8 N/m^2, indicating that the hydration pressure for lysozyme is similar to other biological interfaces such as phospholipid bilayers.

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The Phosphorus-Oxygen Bond As An Intrinsic Vibrational Probe of Electric Field in Biological Systems

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Most proteins lack convenient intrinsic chromophores, and much has been learned by the incorporation of unnatural chromophores such as fluorophores into proteins. However, in some cases the incorporation of these unnatural probes into proteins may unduly influence the properties of the system to be studied. Phosphate compounds are ubiquitous in biology and could serve as intrinsic infrared probes in biological systems such as phospholipid membranes and nucleotide binding proteins. Because vibrational transitions are sensitive to the local electric field through the vibrational stark effect, it is possible to utilize infrared probes to study electric fields. Using model phosphate compounds I have measured the shift in frequency of the phosphate absorption in response to an applied electric field. This sensitivity to electric field is called the stark tuning rate. I am also using the GTPase Ras to measure the stark tuning rate of the nucleotide phosphate vibrations. Once the response of the probe has been calibrated in this way we can measure biologically relevant changes in electric field. For instance, by performing time-resolved infrared absorption measurements on Ras we can study how the electric field in the active site changes during nucleotide hydrolysis. The binding of the GTPase activating protein to Ras results in an increase in the hydrolysis rate of 5 orders of magnitude, and electric field measurements may help explain how this remarkable rate enhancement is brought about.

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Ligand Depletion in vivo Modulates the Dynamic Range of Cooperative Signal Transduction

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A common procedure in systems biology is to build models of physiological networks based on simple experimental measurements made under controlled conditions. One of the frequently performed quantitative measurements is the dose-response relationship. Its results are assumed to be characteristic of the target molecule and independent of contingent controlled variables. However, ligand concentrations *in vivo* are often in the same range as the dissociation constant of their receptors, leading to the phenomenon of ligand depletion. Moreover, biological signaling often involves cooperative interactions for the binding of ligands to their receptors. We show that ligand depletion diminishes cooperativity and broadens the dynamic range of sensitivity to the signaling ligand. The effects are illustrated with the highly cooperative flagella motor of bacteria and with the ubiquitous intracellular calcium-binding molecule, calmodulin. As a result of ligand depletion, the same signal transducer responds to different ranges of signal with various degrees of cooperativity according to its effective cellular concentration. Therefore, results from *in vitro* dose-response properties cannot be directly applied to understand signaling *in vivo*. Moreover, receptor concentration is a key element in controlling signal transduction and its modulation constitutes a way of controlling sensitivity to signals. For quantitative measures of cooperative effects of signaling, the commonly used Hill coefficient assigns misleading values under many conditions. To correct this situation, we define a new index, the Greek letter ''nu'', based on the dose-response cooperativity of any oligomeric receptor with respect to the hypothetical dose-response properties of an ''equivalent monomer''. The index nu provides a robust measure of cooperativity under diverse conditions and reveals that within the context of the Monod-Wyman-Changeux two-state model of cooperative transitions, the true intrinsic cooperativity is equal to the number of ligand-binding subunits in the oligomeric receptor.