Changes in the Lipid Dynamics of Liposomal Membranes Induced by Poly(Ethylene Glycol): Free Volume Alterations Revealed by Inter- and Intramolecular Excimer-Forming Phospholipid Analogs

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ABSTRACT Influence of osmotic shrinkage, swelling, and dehydration on large unilamellar liposomes (LUVs) of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was investigated using the fluorescent lipid probes 1-palmitoyl-2-[10-(pyren-1-yl)]-decanoyl-*sn*-glycero-3-phosphocholine (PPDPC) and 1,2-bis[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phosphocholine (bisPDPC). Increasing concentrations of poly(ethylene glycol) (PEG, average molecular weight of 6000) producing osmotic gradients $\Delta\Omega$ up to 250 mOsm/kg were first added to the outside of LUV labeled with 0.1 mol% of either of the above fluorescent phospholipids. The resulting osmotic shrinkage was accompanied by a progressive reduction in the lateral diffusion of the membrane-incorporated PPDPC, evident as a decrease in the rate of its *intermolecular* excimer formation. In contrast, under the same conditions the rate of *intramolecular* excimer formation by bisPDPC increased. Notably, signals opposite to those described above were observed for both of the fluorescent probes upon osmotic swelling of DOPC liposomes with encapsulated PEG. The lateral diffusion of PPDPC became progressively reduced upon membrane dehydration due to increasing concentrations of symmetrically distributed PEG (with equal polymer concentrations inside and outside of the liposomes) when neither shrinkage nor swelling occurs while enhanced excimer formation by bisPDPC was evident. The latter results were interpreted in terms of osmotically induced changes in the hydration of lipids. In brief, the removal of water from the phospholipid hydration shell diminishes the effective size of the polar headgroup, which subsequently allows for an enhanced lateral packing of the phospholipid acyl chains. Our findings are readily compatible with membrane free volume V_f changes due to osmotic forces under three different kinds of stress (shrinkage, swelling, and dehydration) applied on the lipid bilayers.

INTRODUCTION

Poly(ethylene glycol), PEG, is a nontoxic, water-soluble, and chemically relatively inert synthetic polymer (Malcolm et al., 1957) having a variety of effects on biological systems (Bessler et al., 1977; McCammon and Fan, 1979; Boni and Hui, 1987; Herrmann et al., 1993). It is commonly used as a cell-cell fusogen in the production of somatic hybrids (Roos et al., 1987). However, little is known about the molecular level effects of PEG on cell membranes. PEG has been shown to induce "hemifusion" of phosphatidylcholine liposomes, i.e., lipid mixing without the merging of their aqueous contents (Burgess et al., 1991; Wu et al., 1991). PEG also enhances the lateral packing of phosphatidylcholine and an increase by nearly one degree in the gel→liquid crystalline phase transition temperature of dimyristoyl phosphatidylcholine due to $[PEG]_{OUT}$ = 20%(w/w) has been reported (Maggio et al., 1978; Tilcock et al., 1979; Yamazaki et al., 1992). Dehydration of lipid membranes by PEG has been suggested to be crucial for the ability of this polymer to induce membrane fusion (MacDonald, 1985; Burgess et al., 1992). In addition, osmotic effects are considered to be of importance, and two different mechanisms have been forwarded. The first one involves osmotic shrinkage of liposomes due to an osmotic gradient (Ahkong and Lucy, 1986). The second mechanism is attributed to the exclusion of PEG from the hydration shell adjacent to membrane surface (Arnold et al., 1990). An osmotic imbalance due to osmolyte gradient between the exclusion layer and the bulk PEG solution results in stress that has been considered to be involved in PEG-induced vesicle aggregation (Ito et al., 1989; Yamazaki et al., 1989).

PEG also exerts effects on proteins, which could be relevant for the fusion of cells. Precipitation of soluble proteins and polymerization of cytoskeletal proteins by PEG have been demonstrated (Atha and Ingham, 1981; Lee and Lee, 1981; Arakawa and Timasheff, 1985; Suzuki et al., 1989; Cuneo et al., 1992). Likewise, DNA undergoes a structural transition in solutions containing sufficient concentrations of PEG and salts (Jordan et al., 1972).

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Abbreviations used: PEG, poly(ethylene glycol) with an approximate molecular weight of 6000; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; PPDPC, 1-palmitoyl-2-[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phosphocholine; bisPDPC, 1,2-bis[10-(pyren-1-yl)]decanoyl-*sn*-glycero-3-phosphocholine; V_f, membrane free volume; $[PEG]_{IN}$, $[glucose]_{IN}$, and $[dextran]_{IN}$, the concentrations of these solutes encapsulated in liposomes; $[PEG]_{OUT}$, $[glucose]_{OUT}$, and $[dextran]_{OUT}$, the concentrations of these solutes added to the outside of the liposomes; $[PEG]_{SYM}$, $[glucose]_{SYM}$, and $[dextran]_{SYM}$, the concentrations of these solutes symmetrically distributed on both sides of the liposomal membrane; Δ[PEG], Δ[glucose], and Δ[dextran], the difference in the concentrations of these solutes between the inside and the outside of the liposomes; I_E, pyrene excimer fluorescence intensity at 480 nm; I_M, pyrene monomer fluorescence intensity at 394 nm; LUV, large unilamellar vesicles; Ω, osmotic pressure; $\Delta\Omega$, osmotic pressure gradient.

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The addition of PEG outside the liposomes causes an osmotically driven water efflux across the bilayer resulting in the shrinkage of the vesicles (Burgess and Lentz, 1993). Instead, swelling of the vesicles due to an inward osmotic water flow is produced by first encapsulating PEG into liposomes and subsequently transferring them into a medium with no PEG (Haines et al., 1987). PEG binds water with high affinity, and it has been used to dehydrate phospholipid membranes (Rand and Parsegian, 1989). The removal of water from the hydration shell of the lipid diminishes the effective size of the headgroup, creating compression of the membrane (Rand and Parsegian, 1989). An increasing extent of dehydration of phospholipid liposomes is achieved when LUV are formed in the presence of increasing and equal concentrations of PEG both inside and outside of the liposomes ($[PEG]_{SYM} = [PEG]_{OUT} = [PEG]_{IN}$), i.e., in the absence of a transmembrane osmolyte gradient.

In this study, we provide evidence for poly(ethylene glycol)-induced changes in the membrane free volume as measured by the dipyrenyl phospholipid analog, bisPDPC (Sunamoto et al., 1980), and also revealed by the altered rates of lateral diffusion of the monopyrenyl compound, PPDPC (Galla and Sackmann, 1974). PEG does not interact with phosphatidylcholine membranes (Arnold et al., 1990). To exclude possible effects specific for PEG, we used dextran and glucose.

MATERIALS AND METHODS

Materials

PPDPC and bisPDPC were purchased from K&V Bioware (Espoo, Finland). Dextran (an average molecular weight of 9300) and DOPC were from Sigma Chemical Co. (St. Louis, MO). PEG with an average molecular weight of 6000 was from Fluka (Bubendorf, Switzerland) and was used without further purification (Yamazaki et al., 1989; Burgess et al., 1991). No impurities were detected in the lipids upon thin layer chromatography on silicic acid-coated plates (Merck, Darmstadt, Germany) using chloroform/methanol/water (65:25:4 v/v) as a solvent system and examination of the plates for pyrene fluorescence or after iodine staining.

The concentration of DOPC was determined by phosphorus (Bartlett, 1959). Concentrations of the mono- and dipyrenyl probes were determined spectrophotometrically at 342 nm using 42,000 cm^{-1} as the molar extinction coefficient for pyrene.

Liposome preparation

Lipids were dissolved in chloroform. After mixing of the desired lipid composition the solvent was removed under a stream of nitrogen. The dry lipid mixture was then kept under reduced pressure overnight and subsequently hydrated at a concentration of 30 mM in 2 mM TES, 1 mM EDTA, and 100 mM NaCl, pH 7.4. The given concentrations of PEG, dextran, or glucose were also present when indicated. Hydration was done at 23°C, well above the gel→liquid crystalline transition temperature of DOPC. Unless otherwise stated, the amount of the fluorescent phospholipids was maintained constant to yield 0.1 mol% of total phospholipid. To obtain LUVs, the dispersions were extruded through Nucleopore (Pleasanton, CA) 0.1 μm pore size polycarbonate filters using a LiposoFast low pressure homogenizer (Avestin, Ottawa, Canada) essentially as described (MacDonald et al., 1991). Because of the technique used for the preparation of LUV, we do not expect major differences in their sizes. Possible minor size differences are unlikely to cause major changes in the hydration properties of LUV. Unilamellar vesicles were used because multilamellar vesicles would not allow us to differentiate between osmotic shrinkage and dehydration, for the relatively large PEG molecules are not trapped between the lipid lamellae (Rand and Parsegian, 1990). Before the use of LUV in fluorescence measurements, the final concentration of lipid was in all experiments adjusted to 30 μM.

Osmotic pressure

Three different kinds of experiments were carried out. First, osmotic shrinkage of LUV was induced by mixing liposomes with solutions of indicated osmolarity. In previous studies using DOPC liposomes and $[PEG]_{OUT}$ up to 20% (w/w), no leakage of the contents of the polymer was observed (Burgess et al., 1991). Second, swelling was induced by preparing liposomes in osmolyte-containing buffers and subsequently transferring them into buffers with smaller [osmolyte] to result in an osmotic pressure gradient and water influx. More specifically, lipids were first hydrated (see above) with buffers containing a fixed osmolyte concentration and subsequently extruded through polycarbonate membranes as described above. Thereafter, these LUV were transferred to buffers with different concentrations of PEG to have $\Delta[PEG] = [PEG]_{IN} - [PEG]_{OUT} \geq 0$. The residual amounts of the more concentrated osmolyte solutions used for encapsulation were not removed from the outside of the liposomes. Due to dilution, the resulting systematic error in the value for ΔPEG was maximally only 0.1% (w/w). In the third kind of experiments, dehydration of LUV was accomplished by preparing liposomes in solutions containing the given osmolytes at equal concentrations both the inside and outside of the vesicles. When indicated, dextran or glucose were used instead of PEG. Osmolarities were measured using a freezing point osmometer (Advanced microosmometer, Model 3Mo, MA).

Fluorescence measurements

Fluorescence measurements were carried out with an SLM 4800S spectrofluorometer (SLM Instruments Inc., Urbana, IL). Excitation wavelength of 344 nm as well as pyrene monomer fluorescence at 394 nm were selected by monochromators while the excimer emission was monitored simultaneously with a 470 nm long pass filter. Slits of 1 and 16 nm were used for the excitation and for monomer emission beams. Liposome (2 ml) solution (60 nmol of lipid) was placed in a magnetically stirred four-window quartz cuvette in a holder thermostated at 23°C. Weak fluorescence due to PEG was observed both in the monomer and excimer emission wavelengths upon excitation at 344 nm. Because of the low pyrene concentrations used, this background arising from PEG was subtracted from the measured emission intensities before the calculation of the excimer to monomer fluorescence ratio (I_e/I_m). No evidence for energy transfer from pyrene to PEG or vice versa was observed. To be able to express the numeric data in a more easily handling range and to allow for an easier comparison of the different measurements, the I_e/I_m values used in illustrations were normalized so that the I_e/I_m for 0.1 mol% of PPDPC incorporated in DOPC LUV in the buffer was assigned to 1, and all other values were calculated in relation to this. Intensities shown were not corrected for instrument response. To obtain I_e/I_m values corrected for instrument response and for normalization, the given values should be divided by 30.14. For more facile experimentation, fluorescence measurements were conducted in the presence of atmospheric oxygen (Mustonen and Kinnunen, 1993; Mustonen et al., 1993).

To avoid scattering arising from air bubbles, fluorescence intensities were measured 10 min after the mixing of LUV with PEG, dextran, or glucose solutions. The fluorescence signals were stable for at least 30 min. In keeping with previous studies, the aggregation of liposomes should be minimal at the lipid concentrations used (Tilcock and Fisher, 1982). Furthermore, PEG concentrations were below those required for fusion (Massenburg and Lentz, 1993). Dextran and glucose do not induce fusion of large unilamellar vesicles.

RESULTS

Effects of osmotic shrinkage

Liposomal membranes are permeable to water but not to larger hydrophilic molecules. The presence of increasing concentrations of osmotically active substances, such as PEG, added to the outside of LUV thus results in their osmotic shrinkage (Bangham et al., 1967; Bittman and Blau, 1972; Boroske et al., 1981). Examination of osmotically shrunken LUV revealed that the I_e/I_m values for the intramolecular excimer forming probe bisPDPC in these vesicles increased (Fig. 1 *A*). The increase in the I_e/I_m for bisPDPC depended on $[PEG]_{OUT}$ and was maximally ≈1.55-fold at a polymer concentration of 15% (w/w), corresponding to 250 mOsm/kg. In contrast to bisPDPC, I_e/I_m for the intermolecular excimer-forming phospholipid analog, PPDPC decreased in response to increasing $[PEG]_{OUT}$ (Fig. 1 *A*). The decrease of I_e/I_m was found to depend on $[PEG]_{OUT}$ and was maximally 30% at $\Delta\Omega$ = 250 mOsm/kg.

To exclude the involvement of possible PEG-specific interactions with phosphatidylcholine, the effects of dextran and glucose were also studied. Although of somewhat smaller amplitude, the shrinkage of LUV with $[dextran]_{OUT}$ caused, similarly to PEG, opposite changes to PPDPC and bisPDPC (Fig. 1 *B*). The I_e/I_m values for PPDPC decreased by ≈11% and those for bisPDPC increased by 7% at $[dextran]_{OUT}$ producing $\Delta\Omega$ of 200 mOsm/kg. Similar results were measured for both fluorescent probes also upon the increasing $[glucose]_{OUT}$. However, the maximal effects in the concentration range of glucose studied (up to 1.0 M corresponding to 1200 mOsm/kg) were significantly smaller compared to the effects exerted by either dextran or PEG (Fig. 1 *C*).

Nonideal mixing (phase separation) of PPDPC in DOPC caused by the presence of osmolytes could result in an increase in I_e/I_m. Because of the high degree of fluidity of DOPC membranes, this possibility is remote, yet has to be excluded experimentally (Fig. 2). The data shown, i.e., I_e/I_m versus temperature for PPDPC/DOPC LUV at $[PEG]_{OUT}$ = 15% (w/w) clearly reveals a lack of evidence for polymer-induced phase separation under these conditions.

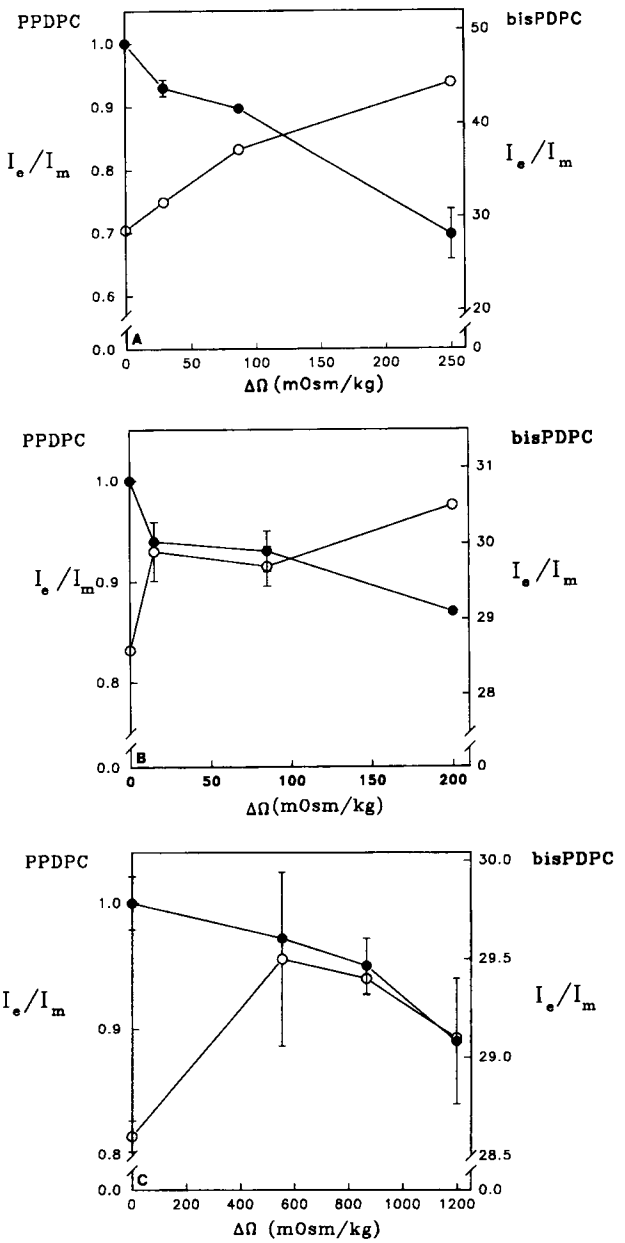

FIGURE 1 (*A*) Changes in the I_e/I_m values for bisPDPC and PPDPC in DOPC LUV upon osmotic shrinkage due to increasing $[PEG]_{OUT}$ (0–15% w/w). The error bars indicate the range of observations for two to six separate experiments. For the sake of clarity, error bars overlapping with symbols are not shown. The I_e/I_m of 0.1 mol% PPDPC (●–●, left scale) in DOPC LUV in buffer was normalized to one, whereas that for 0.1 mol% bisPDPC (○–○, right scale) calculated in proportion to this was 28.6. Buffer was 2 mM TES, 1 mM EDTA, and 100 mM NaCl, pH 7.4. (*B*) Osmotic shrinkage of DOPC LUV due to $[dextran]_{OUT}$ (0–15% w/w) measured with liposomes containing either bisPDPC or PPDPC. (*C*) Same experimental conditions as for panel A but using glucose (0–1 M).

Effects of osmotic swelling

To induce an osmotic water inflow to cause swelling of LUV 10% (w/w), PEG (≈87 mOsm/kg) was first encapsulated in liposomes. Thereafter, these LUV were transferred into solutions of diminishing $[PEG]_{OUT}$ so as to generate a gradient of polymer concentration $\Delta[PEG] = [PEG]_{IN} - [PEG]_{OUT} \geq 0$. The dependencies of the bisPDPC and PPDPC fluorescence signals on the magnitude of the osmotic pressure gradient $\Delta\Omega$ due to $\Delta[PEG]$ are depicted in Fig. 3 *A*. Exactly the opposite behavior was observed for both fluorescent probes upon swelling of the LUV as compared with the effects of osmotic shrinkage described. Accordingly, a decrease in the intramolecular I_e/I_m of bisPDPC was evident upon the increase in $\Delta\Omega$. Instead, an increased intermolecular excimer formation by PPDPC was observed, thus indicating enhanced lateral diffusibility of the latter probe. To compare with PEG, swelling of LUV was also induced using either encapsulated dextran or glucose. Both of these compounds

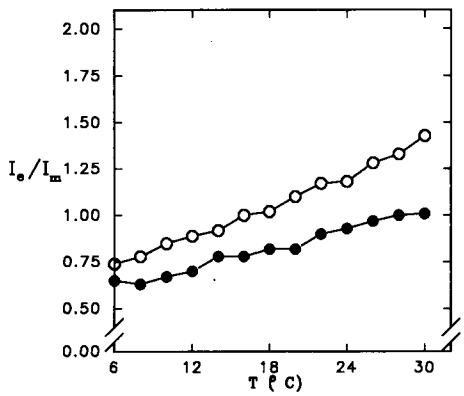

FIGURE 2 Temperature dependency of I_e/I_m for PPDPC incorporated in DOPC LUV. To promote possible phase separation phenomena, the concentration of the probe was increased to 1 mol% of the total lipid. Measurements were made for LUV in the absence of PEG (○–○), and at $[PEG]_{OUT}$ producing an osmotic pressure of 250 mOsm/kg (●–●).

produced fluorescence changes that were qualitatively similar to, although smaller in magnitude than, those due to Δ[PEG] (Fig. 3, *B* and *C*).

Effects of phospholipid dehydration by PEG

Increasing and equal concentrations of PEG on both the inside and the outside of LUV caused qualitatively essentially similar fluorescence changes to those evident for $[PEG]_{OUT}$. In brief, increasing $[PEG]_{SYM}$ induced a progressive increase in the rate of intramolecular excimer formation by bisPDPC, whereas the opposite was evident for the intermolecular I_e/I_m for PPDPC (Fig. 4 *A*). Yet, in the concentration range of this polymer studied (up to 15% (w/w) and corresponding to 250 mOsm/kg), the alterations in I_e/I_m due to $[PEG]_{SYM}$ were larger compared with those caused by identical $[PEG]_{OUT}$. Analogously to PEG, $[dextran]_{SYM}$ reduced the I_e/I_m of PPDPC more efficiently than an equal $[dextran]_{OUT}$. In contrast to PEG-induced fluorescence changes, however, the increase in the intramolecular I_e/I_m of bisPDPC by increasing $[dextran]_{OUT}$ was not exceeded by the fluorescence changes induced by $[dextran]_{SYM}$ (Fig. 4 *B*). Compared with both PEG and dextran, the responses of bisPDPC and PPDPC caused by increasing $[glucose]_{SYM}$ differed. The I_e/I_m values for bisPDPC were not altered by increasing $[glucose]_{SYM}$, whereas the rate of intermolecular excimer formation by PPDPC decreased under these conditions more efficiently than upon increasing $[glucose]_{OUT}$ (Fig. 4 *C*).

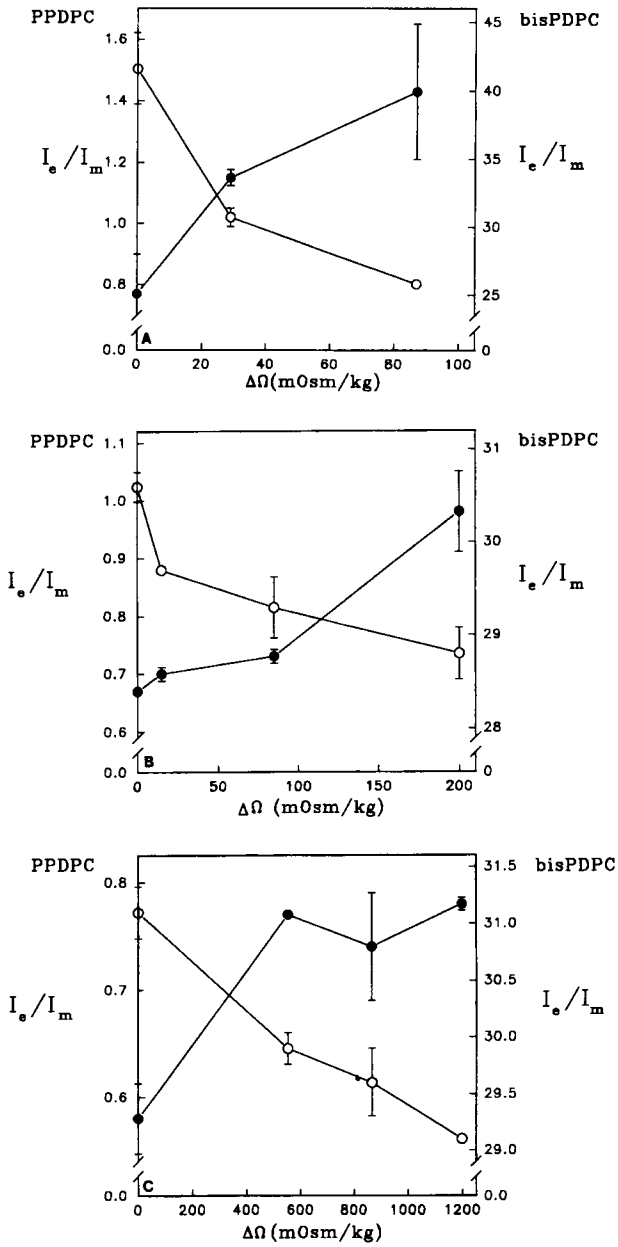

FIGURE 3 (*A*) Osmotic swelling of DOPC LUV due to Δ[PEG]. PEG (10% w/w) was first encapsulated in DOPC LUV (corresponding to 87 mOsm/kg), which were subsequently transferred into solutions of decreasing $[PEG]_{OUT}$. Both bisPDPC (○–○) and PPDPC (●–●) fluorescence signals were measured. Experimental conditions and labels for the graphs are as described for Fig. 1. (*B*) Osmotic swelling of liposomes due to Δ[dextran]. Dextran (corresponding to 200 mOsm/kg, 15% w/w) was encapsulated in DOPC LUV containing either bisPDPC or PPDPC. Thereafter, these liposomes were transferred into solutions of varying $[dextran]_{OUT}$. (*C*) Same experimental conditions as for *A*, but using 1 M glucose (1200 mOsm/kg) encapsulated in liposomes and varying $[glucose]_{OUT}$.

DISCUSSION

A monomeric excited state pyrene relaxes to ground state by emitting photons with a maximum wavelength at ≈394 nm, the exact peak energy and spectral fine structure depending on the solvent polarity. During its lifetime, the excited state pyrene may form a characteristic complex, excimer (excited dimer) with a ground state pyrene. This complex relaxes back to two ground state pyrenes by emitting quanta as a broad and featureless band centered at ≈480 nm. Pyrene-labeled phospholipids are widely employed as membrane probes (for a brief review, see Kinnunen et al., 1993a). Lipid fluorophores containing one pyrene moiety have been used to study the lateral mobility (Galla and Sackmann, 1974; Galla and Hartmann, 1980; Hresko et al., 1986) and distribution of lipids

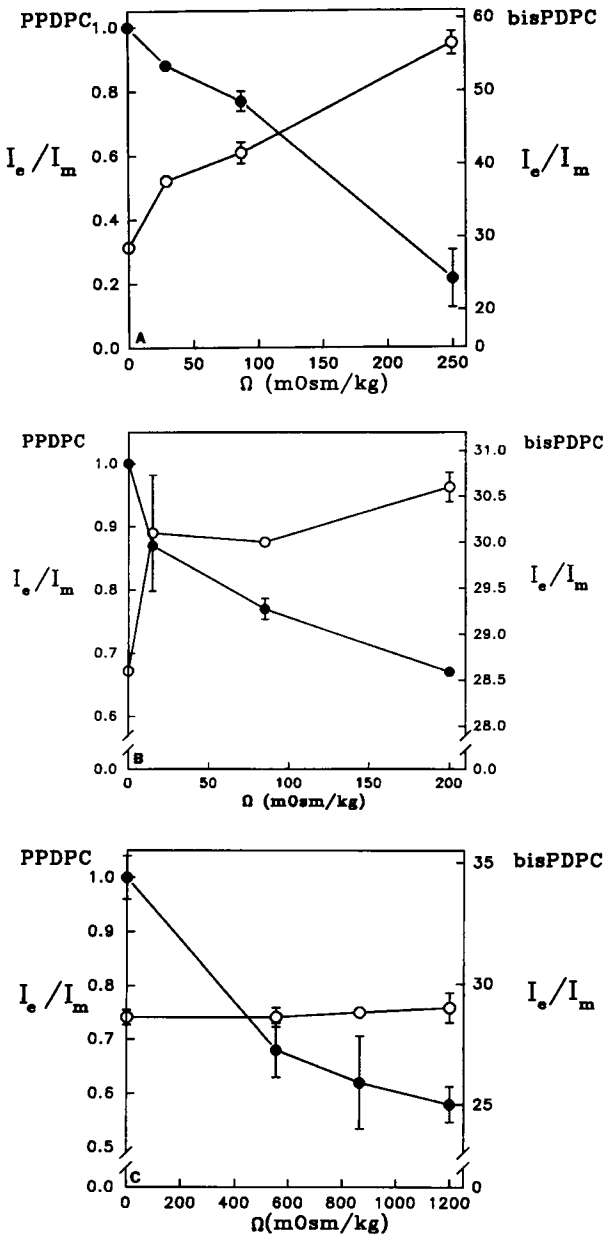

FIGURE 4 (*A*) The response in I_e/I_m values of bisPDPC (○–○) and PPDPC (●–●) in DOPC LUV in the presence of increasing $[PEG]_{SYM}$ (0–15% w/w). Liposomes were prepared in solutions with the indicated concentrations of PEG so as to have no polymer concentration gradient across the membrane. Experimental conditions are otherwise as described for Fig. 1. (*B*) Changes in the I_e/I_m of bisPDPC (○–○) PPDPC (●–●) upon increasing $[dextran]_{SYM}$ (0–15% w/w). Experimental conditions are otherwise as described for Fig. 2. (*C*) Similar experimental conditions as for *A* but using glucose as an osmolyte (0–1 M).

(Somerharju et al., 1985; Eklund et al., 1988; Tang and Chong, 1992) as well as the interdigitated phase (Komatsu and Rowe, 1991) and equilibrium lateral pressure in liposomes (Thurén et al., 1986; Konttila et al., 1988). In addition, we have used these probes in fluorescence resonance energy transfer measurements to monitor the membrane attachment of cytochrome c (Mustonen et al., 1987, 1993; Rytömaa et al., 1992), adriamycin (Mustonen and Kinnunen, 1991; 1993), polyribosomes (Kaihovaara et al., 1991), and DNA (Kinnunen et al., 1993b).

In this study we describe the effects of osmotic (i) swelling, (ii) shrinkage, and (iii) dehydration on I_e/I_m values for PPDPC and bisPDPC incorporated in DOPC LUV. Importantly, we wanted to develop a qualitative molecular level understanding of the impact of these osmotic conditions on the liposomal model membranes as revealed in the fluorescence behavior of these probes. *In membranes containing phospholipid analogs with one pyrene-acyl chain (e.g., PPDPC) excimer formation is an intermolecular collisional event occurring inside the membrane hydrocarbon region (Galla and Sackmann, 1974). In contrast, excimer fluorescence from dipyrene phospholipids such as bisPDPC is intramolecular and concentration independent at sufficiently low probe concentrations (Sunamoto et al., 1980).* The observed fluorescence changes could arise, in principle, from macroscopic membrane processes, such as vesiculation taking place during swelling and shrinkage. Yet it is difficult to understand how such effects could influence the fluorescence of probes measuring their immediate vicinity in highly fluid DOPC membranes. Rupture and leakage of contents has not been observed for vesicles exposed to osmotic pressure gradients of up to 1200 mOsm/kg (Mui et al., 1993). Osmotic shrinkage of small unilamellar vesicles by LiCl (Lerebours et al., 1993) does not produce the leakage of contents from these vesicles. Likewise, $[PEG]_{OUT}$ (up to 20% w/w) does not cause the disruption of DOPC LUV in conditions identical to those used in the present study (Burgess et al., 1991). To conclude, when taking into account the above results and the large size of PEG, it is rather unlikely that leakage of the LUV contents was taking place in the course of our experiments. There is somewhat discrepant data regarding the shape of extruded LUV (MacDonald et al., 1991; Mui et al., 1993). Obviously, LUV may adjust to osmotic pressure gradients also by shape changes. However, shape changes alone would be unlikely to produce the observed type of alterations in the fluorescence signals from the two probes. Accordingly, microscopic changes in the membrane are more likely to be involved.

In the absence of phase separation (Fig. 2.), the osmotically induced changes in the fluorescence of PPDPC are caused by altered diffusional dynamics of the probe in DOPC membranes (Galla and Sackmann, 1974). The rate of the intramolecular excimer formation for a probe such as bisPDPC also depends on the orientation of the pyrene moieties (Thurén et al., 1984; Eklund et al., 1992), on the membrane free volume, and on intramolecular thermal motion (Cheng et al., 1991). Taking into account changes in lipid lateral packing and the qualitative changes in the I_e/I_m for bisPDPC, it is unlikely that in the present experimental conditions the osmotically induced changes in the fluorescence emission of this probe would originate from alterations in the alignment of the acyl chains (Thurén et al., 1984; Eklund et al., 1992).

Free volume V_f is the difference between the effective and the van der Waals volumes per molecule (Bondi, 1954; Cohen and Turnbull, 1959; Turnbull and Cohen, 1970). For

a phospholipid membrane V_f arises from the short-lived, mobile structural defects due to *trans→gauche* isomerization of the lipid acyl chains created because of packing constraints as well as by thermal motion (briefly reviewed in Xiang, 1993). The latter is demonstrated in the measurements of the partial specific volumes of membranes as a function of temperature (Nagle and Wilkinson, 1978; Wilkinson and Nagle, 1979). Free volume model has been used to describe the permeability properties of lipid bilayers (Träuble, 1971). Osmotically induced changes in membrane free volume have been correlated to the diffusion rates of electron transferring quinones in mitochondrial and chloroplast membranes (Mathai et al., 1993).

Increasing concentrations of PEG, glucose, or dextran added to the outside of the liposomes cause water efflux through the membrane, thus resulting in a decreased internal volume of the liposomes (Bangham et al., 1967; Bittman and Blau, 1972; Boroske et al., 1981; Carruthers and Melchior, 1983; Deamer and Bramhall, 1986). Osmotic shrinkage also causes an increased lateral packing of phospholipids and, accordingly, a decrease in the membrane free volume V_f. There are different estimates on the magnitudes of increase in the lateral packing arising from the application of either hydrostatic pressure (Braganza and Worcester, 1986; Scarlata, 1991) or osmotic shrinkage of liposomes (Lis et al., 1982a; McIntosh and Simon, 1986). In giant unilamellar vesicles composed of cholesterol and DMPC, osmotic shrinkage induced an increase in the area-to-volume ratio that is compensated after a limit by fission into smaller vesicles. The mechanism of this process has been suggested to be related to a phase separation of the components (Döberainer et al., 1993). Osmotic swelling of DOPC LUV with encapsulated PEG, glucose, or dextran leads to an increased internal volume of the vesicles and the extent of swelling is directly proportional to the magnitude of osmotic gradient (Sun et al., 1986). The estimates for the maximal area increase $(\Delta A + A)/A$ induced by osmotic swelling range from 5% (Haines et al., 1987) up to 25% (Hanz et al., 1986). Fluidization of the membranes upon swelling has been reported earlier (Boronchov and Boronchov, 1979). Shrinkage of vesicles caused progressively attenuated lateral diffusion of PPDPC (decrease in I_e/I_m), whereas the opposite (increase in I_e/I_m) was true for osmotic swelling. These osmotically induced changes in I_e/I_m for PPDPC are compatible with the proposal for membrane free volume limiting the rate of lipid lateral diffusion (Galla et al., 1979; Vaz and Hallmann, 1983; Vaz et al., 1985; King and Marsh, 1986).

Osmotically induced changes in the rate of intramolecular excimer formation by bisPDPC are also easily rationalized in terms of membrane free volume alterations at a constant temperature. Reducing V_f should attenuate the amplitude of the thermal motion of the pyrenedecanoyl chains, i.e., to decrease the extent of their splaying. While the level of thermal excitation remains constant (i.e., the frequency of chain motions is not reduced), the probability of collisional intramolecular excimer formation during the lifetime of the excited state of pyrene increases upon reduction in V_f. Thus, an increase in I_e/I_m is observed. For obvious reasons the opposite signal, i.e., a decrease in I_e/I_m is evident upon an increase in V_f.

Increasing and equal concentrations of dextran or PEG on both the inside as well as the outside of the liposomes lead to the removal of water from phosphatidylcholine membranes in the absence of net water flow across the bilayer. Interestingly, the effects $[PEG]_{SYM}$ on both bisPDPC and PPDPC are qualitatively similar to those due to osmotic shrinkage. The high affinity of water molecules to the phosphatidylcholine headgroup is well established (Jendrasiak and Hasty, 1974; Klose and Stelzner, 1974; Marčelja and Radić, 1976; LeNeveu et al., 1977; Keith et al., 1977; Wilkinson et al., 1977; Cowley et al., 1978; Hauser et al., 1981; Lis et al., 1982b; Gruen et al., 1984; Mellier and Diaf, 1988; Rand et al., 1988; Sen and Hui, 1988; Rand and Parsegian, 1989; Rand et al., 1990). As PEG binds water efficiently and is excluded from the surface of membranes (Arnold et al., 1990), the addition of this polymer leads to partial dehydration of lipid membranes (Arnold et al., 1983; Sen and Hui, 1988) as well as of proteins (Zimmerberg and Parsegian, 1986). Upon hydration of phosphatidylcholine membranes, decreased bilayer order due to the increasing effective polar headgroup size and weakening van der Waals interactions becomes evident (Rand and Parsegian, 1989). PEG has been shown neither to interact directly with phospholipids nor to induce the formation of nonlamellar structures by phosphatidylcholine (Tilcock and Fisher, 1982; Arnold et al., 1983). Analogously to proteins (Rand, 1992), dehydration of membranes results in a tighter lateral packing of membrane phospholipids due to the reduced effective size of the polar headgroups, which then allows for augmented van der Waals interactions. Accordingly, V_f decreases upon dehydration that should result in lower rates of lateral diffusion (Galla et al., 1979). Removal of water has been reported to attenuate lateral diffusion in partially hydrated egg phosphatidylcholine membranes (McCown et al., 1981). Notably, increasing $[PEG]_{OUT}$ induces, in addition to osmotic shrinkage, dehydration of membrane phospholipids. In the presence of increasing $[PEG]_{SYM}$, phospholipids appear, however, to become more efficiently dehydrated as is evidenced by the enhanced intramolecular excimer formation by bisPDPC, reflecting more effective reduction in V_f. X-ray diffraction studies on unilamellar phospholipid vesicles have revealed an abrupt change from diffuse to constant diffraction patterns when $[PEG]_{OUT}$ exceeds 10% (w/w). These results were obtained at relatively high lipid concentrations and were interpreted as the stacking of aggregated discoid vesicles. Below 10% (w/w) of $[PEG]_{OUT}$, there should be no major deformation of DOPC vesicles, whereas a looser lateral packing (and, thus, increase in local V_f) in the high curvature region of the discoid vesicles (at $[PEG]_{OUT} > 10\%$ w/w) should be evident (Burgess et al., 1992). However, the influence of possible deformation of vesicles on V_f appears to be minor because our results show that the effects of PEG on the behavior of both probes in DOPC LUV to be continuous as a function of $[PEG]_{OUT}$ in the concentration range

from 0 up to 15% (w/w). The concentrations of PEG used in this study do not lead to the leakage of contents and, therefore, even in the highly curved regions there should be no disruption of the lateral packing. As the discoidal DOPC LUV have been proposed to form due to water outflow, one could expect that in the presence of $[PEG]_{SYM}$ vesicles should remain spherical as there is no net water flow through the liposomal membrane. Part of the difference between the effects by $[PEG]_{OUT}$ and $[PEG]_{SYM}$ could arise from this hypothetical difference in shape, i.e., packing disruption in the highly curved regions of these vesicles might increase the free volume as at the same time the dehydration of phospholipid headgroups had the opposite effect. Yet, this possibility is not consistent with our data because $[PEG]_{SYM}$ is more efficient in decreasing the free volume than $[PEG]_{OUT}$ at all polymer concentrations, i.e., even below $[PEG]_{OUT}$ proposed to induce shape changes in liposomes. Due to increasing $\Delta[PEG] = [PEG]_{IN} - [PEG]_{OUT} \geq 0$ liposomes swell because of osmotic water flow into the liposomes, which then causes the membrane area to increase. Obviously, the force producing swelling and lateral expansion of the membrane appears to exceed the counteracting condensing force caused by dehydration of membrane lipids by PEG under these conditions.

Despite the qualitative similarities, there were also certain quantitative differences between the effects exerted by PEG and dextran, thus revealing their mechanisms of action to be different (Table 1). In principle, both dextran and PEG as well as smaller osmotically active substances, should be able to dehydrate the bilayer membranes. When added to phosphatidylcholine liposomes, only PEG causes their aggregation and fusion. Interestingly, dialysis of the vesicles against osmolyte leads to aggregation and fusion of liposomes independent of the osmolyte (MacDonald, 1985). Osmotic shrinkage by dextran increases the I_e/I_m value for bisPDPC to 30.5 and causes ≈11% reduction in the intermolecular excimer formation by PPDPC. Under similar osmotic conditions, the corresponding numbers were 41 and 25% when PEG was used. Symmetrically distributed dextran is rather inefficient in reducing V_f (revealed by the quantitatively similar increase in I_e/I_m for bisPDPC as due to $[dextran]_{OUT}$). Instead, $[dextran]_{SYM}$ efficiently reduces the lateral diffusion of PPDPC, reflected in the 30% decrease in I_e/I_m for this probe. These differences between PEG and dextran are likely to be accounted for the interaction of the latter polymer with phosphatidylcholine (Minetti et al., 1979; Evans and Metcalfe, 1984; Massenburg and Lentz, 1993).

TABLE 1 Quantitative comparison of the effects due to PEG and dextran on DOPC LUV containing PPDPC or bisPDPC (for details, see Discussion)

		I_e/I_m	
		PPDPC	bisPDPC
Stress-free	—	1.0	28.6
Osmotic shrinkage	ΔΩ		
$[PEG]_{OUT}$	250	.70	44
$[dextran]_{OUT}$	200	.88	30.5
Dehydration	Ω		
$[PEG]_{SYM}$	250	.35	57
$[dextran]_{SYM}$	200	.70	30.6
Osmotic swelling	ΔΩ		
Δ[PEG]*	90	1.4 (0.8)	25.8 (41.0)
Δ[dextran]‡	200	1.0 (0.7)	28.5 (30.6)

* $[PEG]_{IN}$ = 10% (w/w), $[PEG]_{OUT}$ = 0. Number in parentheses gives I_e/I_m at $[PEG]_{SYM}$ = 10% (w/w).

‡ $[dextran]_{IN}$ = 15% (w/w), $[dextran]_{OUT}$ = 0. Number in parentheses give I_e/I_m at $[dextran]_{SYM}$ = 15% (w/w).

Glucose is not excluded from the surface of membrane but has been shown to interact with the polar headgroups of phosphatidylcholine. Therefore, it is not expected to dehydrate the membrane surface as efficiently as PEG does (Crowe et al., 1984, 1988; Rudolph et al., 1990; Abrams and Yager, 1993; Rand et al., 1993). The I_e/I_m values for PPDPC in LUV decrease only slightly due to $[glucose]_{OUT}$, whereas increasing $[glucose]_{SYM}$ reduces the lateral diffusion of PPDPC more efficiently than $[glucose]_{OUT}$. Importantly, although the I_e/I_m for bisPDPC in DOPC LUV is increased with osmotic shrinkage due to increasing $[glucose]_{OUT}$, the I_e/I_m of this probe is not altered upon increasing $[glucose]_{SYM}$. These differing effects due to $[glucose]_{SYM}$ and $[glucose]_{OUT}$ thus deviate from those observed for the polymers, PEG and dextran. The lack of proper dehydration and direct interaction with phosphatidylcholine by $[glucose]_{SYM}$ can explain the unchanged V_f and efficiently reduced lateral diffusion of PPDPC under these conditions.

The results presented here concern the properties of LUV composed of phosphatidylcholine with two unsaturated chains. Elucidation of the possible biological implications of membrane free volume changes due to hydration and osmotic forces warrants further studies (Ertel et al., 1993; Mui et al., 1993). In the present context, it is of interest that both hypo- and hyperosmotic shocks induce changes in plant cell metabolism (Maeda and Thompson, 1986; Einspahr et al., 1988), as well as in the function of alveolar macrophages (Lewinston et al., 1976). Osmotic stress has been shown to participate in the regulation of mechanically activated ion channels (Martinac et al., 1987; Sackin, 1989; Alexandre and Lassalles, 1991; Rayner et al., 1992; Oliet and Bourque, 1993) that take part in the controlling of ion homeostasis and regulation of volume of cells. Importantly, the lipid composition of the membrane could modulate the response of transmembrane ion channels to osmotic signals (Martinac et al., 1990). Cells have specific mechanisms for the restoration of their volume after osmotic shocks, involving tyrosine kinase activation (Tilly et al., 1993). Hormonally induced cell volume changes have been suggested to be involved in the regulation of protein degradation and synthesis (von Dahl et al., 1991; Häussinger and Lang, 1991). Interestingly, the enhanced activity of ornithine decarboxylase in osmotically swelled cells is at least partially related to enhanced protein synthesis of this protein yet in the absence of a change in its mRNA production (Perry and Oka, 1980; Poulin and Pegg, 1990). Hyposmotic swelling induces stimulation of adenyl cyclase (Watson, 1990). Exocytosis as

well as fusion of liposomes with planar phospholipid membranes is sensitive to osmotic forces (Cohen et al., 1982; Hampton and Holz, 1983; Pollard et al., 1984). The membrane capacitance of auditory outer hair cells is affected by membrane stress (Iwasa, 1993). Transcription of specific genes in bacteria, as well as in mammalian cardiomyocytes, is enhanced by hyperosmotic shock (Csonka, 1989; Brewster et al., 1993; Wollnik et al., 1993). In bacteria there are specific proteins capable of detecting changes in medium osmolarity (Parkinson, 1993).

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REFERENCES

Abrams, S. B., and P. Yager. 1993. Perturbation of the chain melting transition of DPPC by galactose, agarose and Laurdan as determined by differential scanning calorimetry. *Biochim. Biophys. Acta.* 1146: 127–135.

Ahkong, Q. F., and J. A. Lucy. 1986. Osmotic forces in artificially induced cell fusion. *Biochim. Biophys. Acta.* 858:206–216.

Alexandre, J. A., and J-P. Lassalles. 1991. Hydrostatic and osmotic pressure activated channel in plant vacuole. *Biophys. J.* 60:1326–1336.

Arakawa, T., and S. N. Timasheff. 1985. Mechanism of poly(ethylene glycol) interaction with proteins. *Biochemistry.* 24:6756–6762.

Arnold, K., L. Pratsch, and K. Gawrisch. 1983. Effect of poly(ethylene glycol) on phospholipid hydration and polarity of the external phase. *Biochim. Biophys. Acta.* 728:121–128.

Arnold, K., O. Zschoernig, D. Bartel, and W. Herold. 1990. Exclusion of poly(ethylene glycol) from liposome surfaces. *Biochim. Biophys. Acta.* 1022:303–310.

Atha, D. H., and K. C. Ingham. 1981. Mechanism of precipitation of proteins by poly(ethylene glycol)s. *J. Biol. Chem.* 256:12108–12117.

Bangham, A. D., J. De Gier, and G. D. Greville. 1967. Osmotic properties and water permeability of phospholipid liquid crystals. *Chem. Phys. Lipids.* 1:225–246.

Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* 234:466–468.

Bessler, W. G., L. Schimmelpfeng, and J. H. Peters. 1977. Potentiation of mitogen-induced lymphocyte stimulation by poly(ethylene glycol)s. *Biochem. Biophys. Res. Commun.* 76:1253–1260.

Bittman, R., and L. Blau. 1972. The phospholipid-cholesterol interaction. Kinetics of water permeability in liposomes. *Biochemistry.* 25: 4831–4839.

Bondi, A. 1954. Free volumes and free rotation in simple liquids and liquid saturated hydrocarbons. *J. Phys. Chem.* 58:929–939.

Boni, L. T., and S. W. Hui. 1987. The mechanism of poly(ethylene glycol)-induced fusion in model membranes. *In* Cell Fusion. A. E. Sowers, editor. Plenum Press, New York. 301–330.

Boronchov, A., and H. Boronchov. 1979. Increase in membrane fluidity in liposomes and plant protoplasts upon osmotic swelling. *Biochim. Biophys. Acta.* 550:546–549.

Boroske, E., M. Elwenspoek, and W. Helfrich. 1981. Osmotic shrinkage of giant egg-lecithin vesicles. *Biophys. J.* 34:95–109.

Brewster, J. L., T. de Valoir, N. D. Dwyer, E. Winter, and M. C. Gustin. 1993. An osmosensing signal transduction pathway in yeast. *Science.* 259:1760–1763.

Burgess, S. W., D. Massenburg, J. Yates, and B. R. Lentz. 1991. Poly-(ethylene glycol)-induced lipid mixing but not fusion between synthetic phosphatidylcholine large unilamellar vesicles. *Biochemistry.* 30: 4193–4200.

Burgess, S. W., T. J. McIntosh, and B. R. Lentz. 1992. Modulation of poly(ethylene glycol)-induced fusion by membrane hydration: importance of interbilayer separation. *Biochemistry.* 31:2653–2661.

Butko, P., and K. H. Cheng. 1992. Activation energy and entropy for intramolecular excimer formation in a dipyrenylphosphatidylcholine probe in lamellar and hexagonal lipid phases. *Chem. Phys. Lipids.* 62:39–43.

Carruthers, A., and D. L. Melchior. 1983. Studies of the relationship between bilayer water permeability and bilayer physical state. *Biochemistry.* 22:5797–5807.

Chen, K. H., S-Y. Chen, P. Butko, B. W. Van Der Meer, and P. Somerharju. 1991. Intramolecular excimer formation of pyrene-labeled lipids in lamellar and inverted hexagonal phases of lipid mixtures containing unsaturated phosphatidylcholine. *Biophys. Chem.* 39:137–144.

Cohen, F. S., M. H. Akabas, and A. Finkelstein. 1982. Osmotic swelling of phospholipid vesicles causes them to fuse with a planar phospholipid bilayer membrane. *Science.* 217:458–460.

Cohen, M. H., and D. Turnbull. 1959. Molecular transport in liquids and glasses. *J. Chem. Phys.* 31:1164–1169.

Cowley, A. C., N. L. Fuller, R. P. Rand, and V. A. Parsegian. 1978. Measurement of repulsive forces between charged phospholipid bilayers. *Biochemistry.* 17:3163–3168.

Crowe, J. H., M. A. Whittam, D. Chapman, and L. M. Crowe. 1984. Interactions of phospholipid monolayers with carbohydrates. *Biochim. Biophys. Acta.* 769:151–159.

Crowe, J. H., L. M. Crowe, J. F. Carpenter, A. S. Rudolph, C. A. Wistrom, B. J. Spargo, and T. J. Anchordoguy. 1988. Interactions of sugars with membranes. *Biochim. Biophys. Acta.* 947:367–384.

Csonka, L. N. 1989. Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Rev.* 53:121–147.

Cuneo, P., E. Magri, A. Verzola, and E. Grazi. 1992. "Macromolecular crowding" is a primary factor in the organization of the cytoskeleton. *Biochem. J.* 281:507–512.

von Dahl, S., C. Hallbrucker, F. Lang, and D. Häussinger. 1991. Regulation of cell volume in the perfused rat liver by hormones. *Biochem. J.* 280: 105–109.

Döberainer, H.-G., F. Käs, D. Noppl, I. Sprenger, and E. Sackmann. 1993. Budding and fission of vesicles. *Biophys. J.* 65:1396–1403.

Einspahr, K. J., M. Maeda, and G. A. Thompson, Jr. 1988. Concurrent changes in *Dunaliella salina* ultrastructure and membrane phospholipid metabolism after hyperosmotic shock. *J. Cell Biol.* 107:529–538.

Eklund, K. K., J. A. Virtanen, P. K. J. Kinnunen, J. Kasurinen, and P. J. Somerharju. 1992. Conformation of phosphatidylcholine in neat and cholesterol-containing liquid-crystalline bilayers. Application of a novel method. *Biochemistry.* 31:8560–8565.

Eklund, K. K., J. Vuorinen, J. Mikkola, J. A. Virtanen, and P. K. J. Kinnunen. 1988. Ca^{2+}-induced lateral phase separation in phosphatidic acid/phosphatidylcholine monolayers as revealed by fluorescence microscopy. *Biochemistry.* 27:3433–3437.

Ertel, A., A. G. Maragoni, J. Marsh, F. R. Hallett, and J. M. Wood. 1993. Mechanical properties of vesicles. 1. Coordinated analyses of osmotic swelling and lysis. *Biophys. J.* 64:426–434.

Evans, E., and M. Metcalfe. 1984. Free energy potential for aggregation of mixed phosphatidylcholine/phosphatidylserine lipid vesicles in glucose polymer (dextran) solutions. *Biophys. J.* 45:715–720.

Galla, H.-J., and W. Hartmann. 1980. Excimer-forming lipids in membrane research. *Chem. Phys. Lipids.* 27:199–219.

Galla, H.-J., W. Hartman, U. Theilen, and E. Sackmann. 1979. On two-dimensional passive random walk in lipid bilayers and fluid pathways in biomembranes. *J. Membr. Biol.* 48:215–236.

Galla, H.-J., and E. Sackmann. 1974. Lateral diffusion in the hydrophobic region of membranes: use of pyrene excimer as optical probes. *Biochim. Biophys. Acta.* 339:103–115.

Gruen, D. W. R., S. Marčelja, and V. A. Parsegian. 1984. Water structure near the membrane surface. *In* Cell surface dynamics. A. S. Perelson, C. DeLisi, and F. W. Wiegel, editors. Marcel Dekker, New York. 59–91.

Haines, T. H., W. Li, M. Green, and H. Z. Cummins. 1987. The elasticity of uniform, unilamellar vesicles of acidic phospholipids during osmotic

swelling is dominated by the ionic strength of the media. *Biochemistry.* 26:5439–5447.

Hampton, R. Y., and R. W. Holz. 1983. Effects of changes in osmolality on the stability and function of cultured chromaffin cells and the possible role of osmotic forces in exocytosis. *J. Cell Biol.* 96:1082–1088.

Hanz, E., A. Cao, J. Escaig, and E. Taillander. 1986. The osmotic response of large unilamellar vesicles studied by quasielastic light scattering. *Biochim. Biophys. Acta.* 862:379–386.

Hauser, H., I. Pascher, R. H. Pearson, and S. Sundell. 1981. Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biophys. Biochem. Acta.* 650:21–51.

Häussinger, D., and F. Lang. 1991. Cell volume in the regulation of hepatic function: a mechanism for metabolic control. *Biochim. Biophys. Acta.* 1071:331–350.

Hresko, R. C., I. P. Sugár, Y. Barenholz, and T. E. Thompson. 1986. Lateral distribution of a pyrene-labeled phosphatidylcholine in phosphatidylcholine bilayers: fluorescence phase and modulation study. *Biochemistry.* 25:3813–3823.

Herrmann, A., M. J. Clague, and R. Blumenthal. 1993. Enhancement of viral fusion by nonabsorbing polymers. *Biophys. J.* 65:528–534.

Ito, T., T. Yamazaki, and S. Ohnishi. 1989. Osmoelastic coupling in biological structures: a comprehensive thermodynamic analysis of the osmotic response of phospholipid vesicles and a reevaluation of the "dehydration force" theory. *Biochemistry.* 28:5626–5630.

Iwasa, K. H. 1993. Effect of stress on the membrane capacitance of the auditory outer hair cell. *Biophys. J.* 65:492–498.

Jendrasiak, G. L., and J. H. Hasty. 1974. The electrical conductivity of hydrated phospholipids. *Biochim. Biophys. Acta.* 348:45–54.

Jordan, C. F., L. S. Lerman, and J. H. Venable. 1972. Structure and circular dichroism of DNA in concentrated polymer solutions. *Nature (Lond.).* 236:67–70.

Kaihovaara, P., E. Raulo, and P. K. J. Kinnunen. 1991. Changes in lipid distribution and dynamics in degranulated rat liver rough endoplastic reticulum due to the membrane attachment of polyribosomes. *Biochemistry.* 30:8380–8386.

Keith, A. D., W. Snipes, and D. Chapman. 1977. Spin-label studies on the aqueous regions of phospholipid multilayers. *Biochemistry.* 16:634–641.

King, M. D., and D. Marsh. 1986. Free volume model for lipid lateral diffusion coefficients. Assessment of the temperature dependence in phosphatidylcholine and phosphatidylethanolamine bilayers. *Biochim. Biophys. Acta.* 862:231–234.

Kinnunen, P. K. J., A. Koiv, and P. Mustonen. 1993a. Pyrene-labelled lipids as fluorescent probes in studies on biomembranes and membrane models. *In* Fluorescence Spectroscopy. O. S. Wolfbeis, editor. Springer-Verlag, Berlin. 159–169.

Kinnunen, P. K. J., M. Rytömaa, J. Lehtonen, A. Koiv, P. Mustonen, and A. Aro. 1993b. Sphingosine mediated membrane association of DNA and reversal by phosphatidic acid. *Chem. Phys. Lipids.* 66:75–85.

Komatsu, H., and E. S. Rowe. 1991. Effect of cholesterol on the ethanol-induced interdigitated gel phase in phosphatidylcholine: use of fluorophore pyrene-labeled phosphatidylcholine. *Biochemistry.* 30:2463–2470.

Lerebours, B., E. Wehrli, and H. Hauser. 1993. Thermodynamic stability and osmotic sensitivity of small unilamellar phosphatidylcholine vesicles. *Biochim. Biophys. Acta.* 1152:49–60.

Lee, J. C., and L. L. Y. Lee. 1981. Preferential solvent interactions between proteins and polyethylene glycols. *J. Biol. Chem.* 256:625–631.

LeNeveu, D. M., R. P. Rand, V. A. Parsegian, and D. Gingell. 1977. Measurement and modification of forces between lecithin bilayers. *Biophys. J.* 18:209–230.

Lentz, B. R., G. F. McIntyre, D. J. Parks, J. C. Yates, and D. Massenburg. 1992. Bilayer curvature and certain amphipaths promote poly(ethylene glycol)-induced fusion of dipalmitoylphosphatidylcholine unilamellar vesicles. *Biochemistry.* 31:2643–2653.

Lewinston, N. J., J. Theodore, and E. D. Robin. Intracellular edema, and dehydration: effects on energy metabolism in alveolar macrophages. *Science.* 191:403–404.

Lis, L. J., M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian. 1982a. Measurement of the lateral compressibility of several phospholipid bilayers. *Biophys. J.* 37:667–672.

Lis, L. J., M. McAlister, N. Fuller, R. P. Rand, and V. A. Parsegian. 1982b. Interactions between neutral phospholipid bilayer membranes. *Biophys. J.* 37:657–666.

Klose, G., and F. Stelzner. 1974. NMR investigations of the interaction of water with lecithin in benzene solutions. *Biochim. Biophys. Acta.* 363: 1–8.

MacDonald, R. I. 1985. Membrane fusion due to dehydration by poly(ethylene glycol), dextran or sucrose. *Biochemistry.* 24:4058–4066.

MacDonald, R. C., R. I. MacDonald, B. M. Menco, K. Takeshita, N. K. Subbarao, and L. R. Hu. 1991. Small-volume extrusion apparatus for preparation of large unilamellar vesicles. *Biochim. Biophys. Acta.* 1061: 297–303.

Maeda, M., and G. A. Thompson, Jr. 1986. On the mechanism of rapid plasma membrane and chloroplast envelope expansion in *Dunaliella Salina* exposed to hypoosmotic shock. *J. Cell Biol.* 102:289–297.

Maggio, B, and J. A. Lucy. 1978. Interactions of water-soluble fusogens with phospholipids in monolayers. *FEBS Lett.* 94:301–304.

Malcolm, G. N., and J. S. Rowlinson. 1957. The thermodynamic properties of aqueous solutions of poly(ethylene glycol), polypropylene glycol and dioxane. *Trans. Faraday Soc.* 53:921–931.

Marčelja, S., and N. Radić. 1976. Repulsion of interfaces due to boundary water. *Chem. Phys. Lett.* 42:129–130.

Martinac, B., J. Adler, and C. Kung. 1990. Mechanosensitive ion channels of E. coli activated by amphipaths. *Nature.* 348:261–263.

Martinac, B., M. Buechner, A. H. Delcour, J. Adler, and C. Kung. 1987. Pressure-sensitive ion channel in Escherichia coli. *Proc. Natl. Acad. Sci. USA.* 84:2297–2301.

Massenburg, D., and B. R. Lentz. 1993. Poly(ethylene glycol)-induced fusion and rupture of dipalmitoylphosphatidylcholine large, unilamellar extruded vesicles. *Biochemistry.* 32:9172–9180.

Mathai, J. C., Z. E. Sauna, O. John, and V. Sitaramam. 1993. Rate-limiting step in electron transport. *J. Biol. Chem.* 268:15442–15454.

McCammon, J. R., and V. S. C. Fan. 1979. Release of membrane constituents following poly(ethylene glycol) treatment of HEp-2 cells. *Biochim. Biophys. Acta.* 551:67–73

McCown, T. J., E. Evans, S. Diehl, and H. G. Wiles. 1981. Degree of hydration and lateral diffusion in phospholipid multibilayers. *Biochemistry.* 20:3134–3138.

McIntosh, Y. J., and S. A. Simon. 1986. Hydration force and bilayer deformation: a reevaluation. 1986. *Biochemistry.* 25:4058–4066.

Mellier, A., and A. Diaf. 1988. Infrared study of phospholipid hydration. Main phase transition of saturated phosphatidylcholine/water multilamellar samples. *Chem. Phys. Lipids.* 46:51–56.

Minetti, M., P. Aducci, and V. Viti. 1979. Interaction of neural polysaccharides with phosphatidylcholine multilamellar liposomes. Phase transitions studied by the binding of fluorescein conjugated dextrans. *Biochemistry.* 18:2541–2548.

Mui, B. L.-S., P. Cullis, E. A. Evans, and T. D. Madden. 1993. Osmotic properties of large unilamellar vesicles prepared by extrusion. *Biophys. J.* 64:443–453.

Mustonen, P., J. A. Virtanen, P. J. Somerharju, and P. K. J. Kinnunen. 1987. Binding of cytochrome c to liposomes as revealed by the quenching of fluorescence from pyrene-labeled phospholipids. *Biochemistry.* 26: 2991–2996.

Mustonen, P., and P. K. J. Kinnunen. 1991. Activation of phospholipase A_2 by adriamycin in vitro: role of drug/lipid interactions. *J. Biol. Chem.* 266:6302–6307.

Mustonen, P., and P. K. J. Kinnunen. 1993. On the reversal by deoxyribonucleic acid of the binding of adriamycin to cardiolipin containing liposomes. *J. Biol. Chem.* 268:1074–1080.

Mustonen, P., J. Lehtonen, A. Koiv, and P. K. J. Kinnunen. 1993. Effects of sphingosine on peripheral membrane interactions: comparison of adriamycin, cytochrome c, and phospholipase A_2. *Biochemistry.* 32:5373–5380.

Müller, H.-J., and H.-J. Galla. 1983. Pressure variation of the lateral diffusion in the lipid bilayer membranes. *Biochim. Biophys. Acta.* 733: 291–294.

Nagle, J. F., and D. A. Wilkinson. 1978. Lecithin bilayers. Density measurements and molecular interactions. *Biophys. J.* 23:159–175.

Newman, G. C., and C. Huang. 1975. Structural studies on phosphatidylcholine-cholesterol mixed vesicles. *Biochemistry.* 14:3363–3370.

Ohno, H., Y. Maeda, and E. Tsuchida. 1981. ^{1}H-NMR study of the effect of synthetic polymers on the fluidity, transition temperature and fusion of dipalmitoyl phosphatidylcholine small vesicles. *Biochim. Biophys. Acta.* 642:27–36.

Oliet, S. H. R., and C. W. Bourque. 1993. Mechanosensitive channels transduce osmosensitivity in supraoptic neurons. *Nature.* 364:341–343.

Parkinson, J. S. 1993. Signal transduction schemes of bacteria. *Cell.* 73: 857–871.

Perry, J. W., and T. Oka. 1980. Regulation of ornithine decarboxylase in cultured mouse mammary gland by the osmolarity in the cellular environment. *Biochim. Biophys. Acta.* 629:24–35.

Pollard. H. B., C. J. Pazoles, C. E. Creutz, J. H. Scott, O. Zinder, and A. Hotchkiss. 1984. An osmotic mechanism for exocytosis from dissociated chromaffin cells. *Biophys. J.* 25:1114–1121.

Poulin, R., and A. E. Pegg. 1990. Regulation of ornithine decarboxylase expression by anisosmotic shock in α-difluoromethylornithine-resistant L1210 cells. *J. Biol. Chem.* 265:4025–4032.

Rand, R. P. 1992. Raising water to new heights. *Science.* 256:618.

Rand, R. P., N. L. Fuller, P. Butko, G. Francis, and P. Nicholls. 1993. Measured change in protein solvation with substrate binding and turnover. *Biochemistry.* 32:5925–5929.

Rand, R. P., N. L. Fuller, S. M. Gruner, and V. A. Parsegian. 1990. Membrane curvature, lipid segregation, and structural transitions for phospholipids under dual-solvent stress. *Biochemistry.* 29:76–87.

Rand, R. P., N. L. Fuller, V. A. Parsegian, and D. C. Rau. 1990. Variation in hydration forces between neutral phospholipid bilayers: evidence for hydration attraction. *Biochemistry.* 27:7711–7722.

Rand, R. P., and V. A. Parsegian. 1989. Hydration forces between phospholipid bilayers. *Biochim. Biophys. Acta.* 988:351–376.

Rayner, M. D., J. G. Starkus, P. C. Ruben, and D. A. Alicata. 1992. Voltage-sensitive and solvent sensitive processes in ion channel gating. *Biophys. J.* 61:96–108.

Roos, D. S., R. L. Davidson, P. W. Choppin. 1987. Control of membrane fusion in poly(ethylene glycol)-resistant cell mutants: application to fusion technology. *In* Cell Fusion. A. E. Sowers, editor. Plenum Press, New York. 123–144.

Rudolph, B. R., I. Chadrasekhar, B. P. Gaber, and M. Nagumo. 1990. Molecular modeling of saccharide-lipid interactions. *Chem. Phys. Lipids.* 53:243–261.

Rytömaa, M., P. Mustonen, and P. K. J. Kinnunen. 1992. Reversible, nonionic, and pH-dependent association of cytochrome c with cardiolipin-phosphatidylcholine liposomes. *J. Biol. Chem.* 31:22243–22248.

Sackin, H. 1989. A Stretch-activated K^+ channel sensitive to cell volume. *Proc. Natl. Acad. Sci. USA.* 86:1731–1735.

Sen, A., and S.-W. Hui. 1988. Direct measurement of headgroup hydration of polar lipids in inverted micelles. *Chem. Phys. Lipids.* 49:179–184.

Somerharju, P. J., J. A. Virtanen, K. K. Eklund, P. Vainio, and P. K. J. Kinnunen. 1985. 1-palmitoyl-2-pyrenedecanoyl glycerophospholipids as membrane probes: evidence for regular distribution in liquid-crystalline phosphatidylcholine bilayers. *Biochemistry.* 24:2773–2781.

Sunamoto, J., H. Kondo, T. Nomura, H. Okamoto. 1980. Liposomal membranes. 2. Synthesis of a novel pyrene-labeled lecithin and structural studies on liposomal bilayers. *J. Am. Chem. Soc.* 102:1146–1152.

Sun, S.-T., A. Milon, T. Tanaka, G. Ourisson, and Y. Nakatani. 1986. Osmotic swelling of unilamellar vesicles by the stopped-flow light scattering method. Elastic properties of vesicles. *Biochim. Biophys. Acta.* 860: 525–530.

Suzuki, A., M. Yamazaki, and T. Ito. 1989. Osmoelastic coupling in biological structures: formation of parallel bundles of actin filaments in a crystalline-like structures caused by osmotic stress. *Biochemistry.* 28: 6513–6518.

Tang, D, and P. L-G. Chong. 1992. E/M dips. *Biophys. J.* 63:903–910.

Taylor, R. P., C-H. Huang, A. V. Broccoli, and L. Leake. 1977. Nuclear magnetic resonance studies of amphiphile hydration. *Arch. Biochem. Biophys.* 183:83–89.

Ter-Minassian-Saraga, L., and G. Madelmont. 1982. Cholesterol-induced modulation of membrane hydration studies by thermal analysis. *FEBS lett.* 137:137–140.

Thurén, T., P. Vainio, J. A. Virtanen, P. Somerharju, K. Blomqvist, and P. K. J. Kinnunen. 1984. Evidence for the control of the action of phospholipases A by the physical state of the substrate. *Biochemistry.* 23: 5129–5134.

Thurén, T., J. A. Virtanen, and P. K. J. Kinnunen. 1986. Estimation of the equilibrium lateral pressure in 1-palmitoyl-2-[6(pyren-1-yl)]hexanoyl-glycerophospholipid liposomes. *Chem. Phys. Lipids.* 41:329–334.

Tilcock, C. P. S., and D. Fisher. 1979. Interactions of phospholipid membranes with poly(ethylene glycol)s. *Biochim. Biophys. Acta.* 577:53–61.

Tilcock, C. P. S., and D. Fisher. 1982. The interaction of phospholipid membranes with poly(ethylene glycol) vesicle aggregation and lipid exchange. *Biochim. Biophys. Acta.* 688:645–652.

Tilly, B. C., N. van den Berghe, L. G. J. Tertoolen, M. J. Edixhoven, and H. R. de Jonge. 1993. Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances. *J. Biol. Chem.* 268:19919–19922.

Träuble, H. 1971. The movement of molecules across lipid membranes: A molecular theory. *J. Membr. Biol.* 4:193–208.

Turnbull, D., and M. H. Cohen. 1970. On the free volume model of the liquid-glass transition. *J. Chem. Phys.* 52:3038–3041.

Vaz, W. L. C., R. M. Clegg, and D. Hallman. 1985. Translational diffusion of lipids in liquid crystalline phase phosphatidylcholine multibilayers. A comparison of experiment with theory. *Biochemistry.* 24:781–786.

Vaz, W. L. C., and D. Hallmann. 1983. Experimental evidence against the applicability of the Saffman-Delbrück model to the translational diffusion of lipids in phosphatidylcholine bilayer membranes. *FEBS Lett.* 152: 287–290.

Watson, P. A. Direct stimulation of adenylate cyclase by mechanical forces in S49 mouse lymphoma cells during hypoosmotic swelling. *J. Biol. Chem.* 265:6569–6575.

Wilkinson, D. A., H. J. Morowitz, and J. H. Prestegard. 1977. Hydration of phosphatidylcholine. Adsorption isotherm and proton nuclear magnetic resonance studies. *Biophys. J.* 20:169–179.

Wilkinson, D. A., and J. F. Nagle. 1979. Dilatometric study of binary mixtures of phosphatidylcholines. *Biochemistry.* 18:4244–4249.

Wollnik, B., C. Kubisch, A. Maass, H. Vetter, and L. Neyses. 1993. Hyperosmotic stress induces immediate-early gene expression in ventricular adult cardiomyocytes. *Biochem. Biophys. Res. Commun.* 194:642–646.

Wu, J. R., and B. R. Lentz. 1991. Mechanism of poly(ethylene glycol)-induced lipid transfer between phosphatidylcholine large unilamellar vesicles: a fluorescent probe study. *Biochemistry.* 30:6780–6787.

Xiang, T.-X. 1993. A Computer simulation of free-volume distributions and related structural properties in a model lipid bilayer. *Biophys. J.* 65:1108–1120.

Yamazaki, M., S. Ohnishi, and T. Ito. 1989. Osmoelastic coupling in biological structures: decrease in membrane fluidity and osmophobic association of phospholipid vesicles in response to osmotic stress. *Biochemistry.* 28:3710–3715.

Yamazaki, M., M. Ohshika, N. Kashiwagi, and T. Asano. 1992. Phase transitions of phospholipid vesicles under osmotic stress and in the presence of ethylene glycol. *Biophys. Chem.* 43:29–37.

Zimmerberg, J., and V. A. Parsegian. 1986. Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel. *Nature.* 323:36–39.