Osmoelastic Coupling in Biological Structures: Decrease in Membrane Fluidity and Osmophobic Association of Phospholipid Vesicles in Response to Osmotic Stress†

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ABSTRACT: Poly(ethylene glycol)- (PEG-) induced change in membrane fluidity and aggregation of phospholipid vesicles were studied. A threshold concentration of PEG was required to induce the aggregation. This concentration increased with a decrease in the molecular weight of PEG, e.g., from 5% (w/w) with PEG 6000 (PEG with an average molecular weight of 7500) to more than 30% (w/w) with PEG 200. The aggregation was reversible upon dilution of PEG if the initial PEG concentration was smaller than a certain value, e.g., 22% (w/w) for PEG 6000. Addition of PEG caused a decrease in membrane fluidity of the vesicles detected by fluorescence anisotropy of diphenylhexatriene and by electron spin resonance of a spin-labeled fatty acid. The anisotropy change of diphenylhexatriene fluidity change had an inflection point at ~5% (w/w) of PEG 6000, which might suggest that the aggregation would make the decrease of membrane fluidity smaller. Transfer of lipid molecules between phospholipid vesicles was enhanced by the PEG-induced aggregation. The enhancement occurred not only upon direct addition of PEG to the suspending medium, but also upon dialysis of the vesicle suspension against a high concentration of PEG. All these features are consistent with osmoelastic coupling in the phospholipid membranes and the subsequent osmophobic association of the vesicles. The imbalance of osmolarity between the region adjacent to the vesicle surface (exclusion layer) and the bulk aqueous phase, which results from the preferential exclusion of PEG from the exclusion layer in the case of direct addition of PEG, exerts an osmotic stress on the vesicles. The osmotic stress would be counterbalanced by an elastic pressure resulting from elastic strain of the membrane, and it would increase the free energy of vesicles in the dispersed state (osmoelastic coupling). When the osmotic stress exceeds a threshold level, the vesicles would aggregate to avoid further increase in the free energy (osmophobic association).

High molecular weight poly(ethylene glycols) (PEGs) are known to cause aggregation of phospholipid vesicles and induce their membrane fusions at relatively high concentrations of PEG (Tilcock & Fisher, 1982; Saez et al., 1982). The phenomena have attracted much attention as a model system for PEG-induced cell–cell fusions, which are widely used to produce somatic cell hybrids (Davidson & Gerald, 1976; Lucy, 1977). The mechanisms of aggregation and fusion, however, are unclear yet. There have been several studies that emphasize the importance of direct interaction of PEG with membrane lipids (Tilcock & Fisher, 1979; Boni et al., 1984). However, MacDonald (1985) has observed that dialysis of lipid vesicle suspension against a solution containing a high concentration of PEG or dextran caused an increase in the light scattering of the suspension and also intermixing of the lipid molecules. This finding indicates that direct interaction of PEG with phospholipids is not essential.

To analyze osmotic behaviors of phospholipid vesicles in response to osmotic stress, we have proposed a novel concept of the osmoelastic coupling in phospholipid membranes (Ito, Yamazaki, and Ohnishi, submitted for publication). It is a mechanochemical coupling between the elasticity of phospholipid membranes and the osmotic stress arising from imbalance of osmolarity. A high molecular weight cosolvent such as PEG may be excluded preferentially from the regioon adjacent to membrane surface (exclusion layer). It causes the local imbalance of osmolarity between the exclusion layer and the bulk aqueous phase. The membrane would, in turn, be osmotically strained since it should create an elastic pressure that counterbalances the osmotic stress arising from the imbalance of the osmolarity (osmoelastic coupling).

In the present paper, we propose a mechanism of PEG-induced aggregation of phospholipid vesicles that is based on the osmoelastic coupling in phospholipid membranes. The PEG-induced osmotic stress may increase the free energy of the vesicles in the dispersed state owing to the elastic strain of the membranes caused by the osmoelastic coupling. Consequently, when the osmotic stress exceeds a threshold level, the vesicles would aggregate tightly to avoid further increase in the free energy (osmophobic association). The proposed mechanism can reasonably explain not only the characteristic features of PEG-induced aggregation of phospholipid vesicles, such as the dependence on PEG molecular weight and the effect of surface charge of vesicles, but also the aggregation induced by dialysis of phospholipid suspension against high concentrations of PEG or dextran.

Materials and Methods

Chemicals. Egg yolk phosphatidylcholine (egg yolk PC) was extracted from egg yolk and purified according to the method of Singleton et al. (1965). Spin-label probes, 5-doxylstearic acid (5-SAL) and 2-(12-doxylstearoyl)phosphatidylcholine (12-PC*), were prepared according to the methods of Hubbell and McConnell (1971). Tempophosphatidylcholine

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(TEMPO-PC) was synthesized from tempocholine chloride and egg yolk PC according to the method of Kornberg and McConnell (1971). A fluorescence probe, phosphatidyl-*N*-(4-nitrobenz-2-oxa-1,3-diazolyl)ethanolamine (NBD-PE) was synthesized from egg yolk phosphatidylethanolamine and 7-chloro-4-nitrobenz-2-oxa-1,3-diazole according to the method of Struck et al. (1981). Diphenylhexatriene (DPH) and octadecylrhodamine (R_{18}) were purchased from Molecular Probes Inc., and dimirystoylphosphatidylcholine (DMPC) was purchased from Sigma Chemical Co. PEG 200, PEG 1000, PEG 4000, PEG 6000, and PEG 20000 were purchased from Nakarai Chemical Ltd. and were used without further purification.

Preparation of Phospholipid Vesicles. Phospholipid vesicles were prepared with a tip type sonicator (Kaijo Denki) as follows. One milliliter of PIPES[1] buffer (140 mM NaCl, 10 mM PIPES, PH 7.5) was added to appropriate amounts of dried phospholipid, or a mixture of phospholipid and the probe molecule. Then the solution was sonicated at 20 mA of the plate current intermittently for 15 min on ice under N_2-saturated atmosphere. The amount of phospholipid was 0.8 mg for both the light scattering and fluorescence measurements and 80 mg for the ESR measurements. After sonication, the suspensions were centrifuged at 10000*g* for 20 min. DPH-labeled egg yolk PC vesicles were prepared by adding 2 μL of 1 mM DPH solution in tetrahydrofuran to 1 mL of the vesicle suspension and then incubating for 1 h at 37 °C.

Light Scattering and Fluorescence Measurements. A Hitachi 850s spectrofluorometer was used for both light scattering and fluorescence measurements.

For light scattering measurements, the phospholipid vesicle suspensions were mixed with appropriate amounts of PEG to obtain a final phospholipid concentration of 0.1 mM and final PEG concentrations of 0–30% (w/w). The wavelength and the angle of scattering were 450 nm and 90°, respectively.

For fluorescence anisotropy measurements, the fluorescence-labeled egg yolk PC vesicle suspensions were mixed with appropriate amounts of PEG to obtain a final phospholipid concentration of 0.1 mM. The molar ratios of egg yolk PC to DPH and to NBD-PE were 500 to 1 and 100 to 1, respectively. The excitation and emission wavelengths were 358 and 429 nm for DPH-labeled vesicles and 463 and 543 nm for NBD-PE-labeled vesicles, respectively. The anisotropy was calculated according to the conventional procedure with 1.19 as the correction factor of diffraction grating for DPH-labeled vesicles and 1.38 for NBD-PE vesicles (Kouyama, 1983).

The transfer rate of R_{18} between the lipid vesicles was assayed according to the method of Hoekstra et al. (1984). The concentration of phospholipid in the assay mixture was 0.1 mM.

ESR Measurements. All of the ESR spectra were measured with a JEOL ME2X ESR spectrometer. For the membrane fluidity measurements, the suspensions of DMPC vesicles labeled with 5-SAL or with TEMPO-PC were mixed with appropriate amounts of PEG, and then the spectra were measured at 34 °C. The final concentration of DMPC was 20 mM, and the molar ratio of DMPC to 5-SAL or to TEMPO-PC was 100 to 1 in either case. For the lipid-transfer measurements, egg yolk PC vesicles labeled with 20 mol % 12-PC* were mixed with pure egg yolk PC vesicles at a molar ratio of 1 to 9. The transfer was estimated from the increase in the central peak of the ESR spectra (Maeda et al., 1975).

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; PIPES, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid).

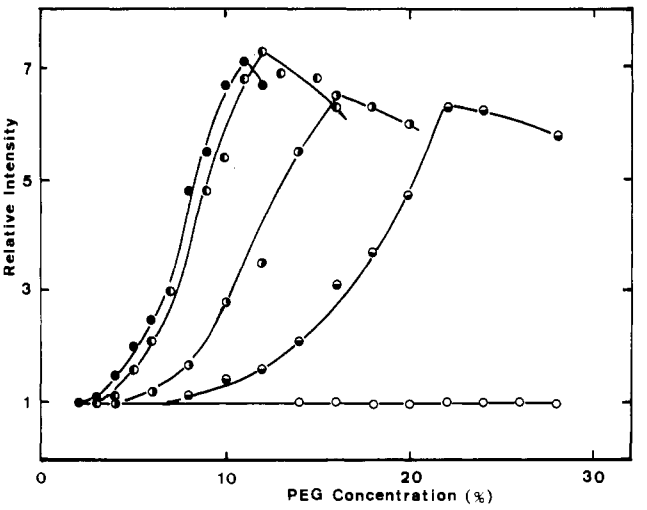

FIGURE 1: Increase in the light scattering of egg yolk PC vesicle suspensions caused by PEGs with different molecular weights: PEG 20000 (●), PEG 6000 (◐), PEG 4000 (◑), PEG 1000 (◒), and PEG 200 (○). The ordinate gives the relative intensity of the light scattering represented as the ratio of the intensity in the presence to that in the absence of PEG.

Table I: Molecular Weight Dependence of the Threshold Concentration and Osmolarity of PEG Causing Aggregation of Egg Yolk PC Vesicles[a]

PEG no.	molecular weight (av)	threshold concn (% w/w)	threshold osmolarity (mosm)
200	200	>30	
1000	1000	11.2	217
4000	3000	7.4	65
6000	7500	5.4	28
20000	20000	4.0	16

[a] The threshold values of the concentration and osmolarity were defined as those values of PEG at which the intensity of the light scattering became 1.5 times as large as that in the absence of PEG.

The final concentration of phospholipid in the assay mixture was 12.5 mM.

Osmolarity Measurements. The osmolarity of PEG at various concentrations was measured at room temperature with a freezing point osmometer (Advance Instrument Inc.).

Results

Increase in Light Scattering Induced by PEG. Addition of PEG caused an increase in the light scattering of a suspension of egg yolk PC vesicles (Figure 1). The increase was strongly dependent on the molecular weight of PEG as observed by Tilcock and Fischer (1982). A higher molecular weight PEG induced the increase at a lower concentration. For example, PEG 6000 caused a detectable increase at a concentration of 5% (w/w), whereas PEG 200 did not, even at 30% (w/w). The threshold concentration and osmolarity of PEG to cause the minimal detectable increase are given in Table I, and the relative value of the inverse of the threshold osmolarity is plotted as a function of the molecular weight of PEG in Figure 2. The dependence of the increase in the light scattering on PEG molecular weight is analyzed by the osmophobic association theory under Discussion.

The PEG-induced increase in the light scattering was reversible; when the vesicle suspension was diluted with PEG-free buffer, the PEG-induced increase in the light scattering vanished as observed by Boni et al. (1984) (Figure 3). The reversibility was complete when the concentration of PEG prior to the dilution was lower than a certain value, 22% (w/w) in the case of PEG 6000, above which the reversibility was

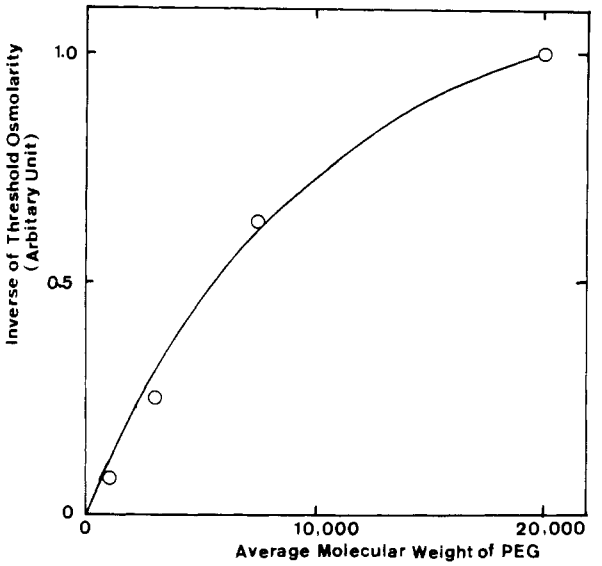

FIGURE 2: Dependence of the threshold osmolarity on the molecular weight of PEG. The inverse value of the critical osmolarity of PEG is plotted against the average molecular weight. The full line represents a theoretical curve based on eq 2, which is analyzed according to the osmophobic association theory. The distribution coefficient K for PEG 20 000 is assumed to be 0.15 in the analysis. See text for the details.

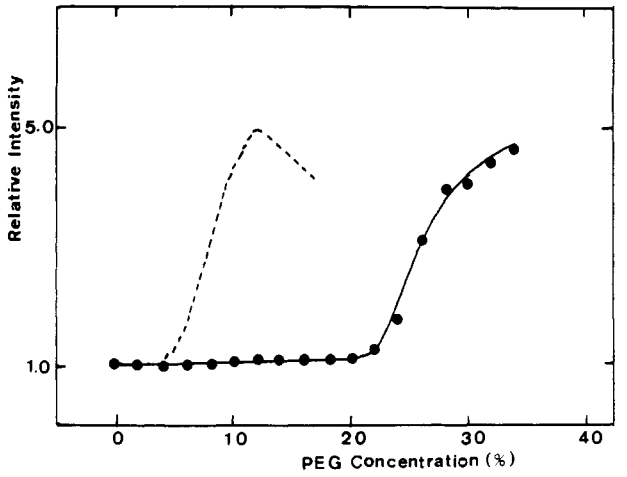

FIGURE 3: Effect of dilution on the PEG-induced increase in the light scattering of egg yolk PC vesicle suspensions. Egg yolk PC vesicle suspensions containing PEG 6000 at various concentrations were diluted 20-fold with PEG-free buffer, and the light scattering was measured. The relative light scattering intensity of the diluted samples was plotted against the concentration of PEG 6000 before the dilution (full line). The dotted line represents the relative light scattering intensity before the dilution as shown in Figure 1.

partial. The reversibility suggests that the increase in the light scattering is due to aggregation of the vesicles, not due to fusions. The observed irreversibility at higher PEG concentrations indicates occurrence of fusions as well. The fusions under such conditions were confirmed by a quick frozen replica electron microscopy (Yamazaki, Ohnishi, Kanazeki, and Ito, unpublished experiments). When negatively charged phospholipid such as phosphatidylserine (PS) or phosphatidic acid (PA) was incorporated into the PC vesicles, the threshold concentration for the PEG-induced aggregation was increased as observed by Boni et al. (1984) (Figure 4). The increase in the threshold concentration was larger with the increase in the negative charge contents. The effect was much enhanced in lower ionic strength media. For example, the threshold of PEG 6000 for the aggregation of vesicles containing 20 mol % PS was 13% (w/w) in 50 mM NaCl, in contrast to 6.4% in 150 mM NaCl. On the other hand, aggregation of pure PC vesicles was independent of the ionic strength.

Table II: Exchange of a Fluorescence Lipid Analogue, R_{18}, between Egg Yolk PC Vesicles Induced by PEG 6000[a]

concn (% w/w)	$t_{1/2}$ (min)	concn (% w/w)	$t_{1/2}$ (min)
10	13.8	25	1.3
15	6.9	30	<0.5
20	3.5		

[a] PEG 6000 at various concentrations was added to a mixture of egg yolk PC vesicles and those containing 5 mol% R_{18}, and the fluoresence intensity at 590 nm with the excitation at 560 nm was measured. The concentration of phospholipid in the assay mixture was 0.1 mM. The time for the intensity to reach a hlaf of the maximum $t_{1/2}$ is given.

Decrease in the Membrane Fluidity Caused by PEG. Addition of high molecular weight PEG to vesicle suspensions caused an increase in the fluorescence anisotropy of DPH incorporated into the vesicle membrane. The anisotropy increased with the increase in the PEG concentration [(a) in Figure 5]. Furthermore, a discontinuity in the rate of increase was observed at around 5% (w/w) of PEG 6000 above which the rate became smaller. This critical concentration agrees with that for the light scattering increase. A low molecular weight PEG 200 did not affect the anisotropy significantly at concentrations up to 10% (w/w). In contrast to DPH, the fluorescence anisotropy of NBD-PE incorporated into the vesicle membrane was not affected upon addition of PEG 6000 [(b) in Figure 5]. As it is known that the mobility of DPH reflects the viscosity of the hydrocarbon region of phospholipid bilayer membranes and that of NBD-PE reflects the viscosity of the polar head region (Shinitzky & Barenholtz, 1978), these results indicate that high molecular weight PEG causes a decrease in the membrane fluidity without affecting the mobility of the polar head group.

The PEG-induced change in the membrane fluidity was also detected by the spin-label 5-SAL, which reflects the viscosity of the hydrocarbon region. The maximum value of the anisotropic hyperfine splitting of 5-SAL incorporated into DMPC vesicles in the liquid-crystalline phase increased with the increasing concentration of PEG 6000 [(a) in Figure 6]. On the other hand, the ESR spectrum of TEMPO-PC, which senses the viscosity of the polar head region, was not changed significantly upon the addition of PEG [(b) in Figure 6].

Exchange of Phospholipid Molecules between Vesicles. The PEG-induced aggregation resulted in transfer of phospholipid molecules between the vesicles. ESR spectra of a mixture of pure PC vesicles and those containing a spin-labeled phospholipid 12-PC* changed on addition of a high molecular weight PEG. The peak height increased with time [(a) in Figure 7]. The increase was observed even at a small concentration of PEG 20 000, 4% (w/w), at which the aggregation started [(a) in Figure 7], but it was not observed at 1% (w/w) [(b) in Figure 7].

The dependence of the lipid transfer on the concentration of PEG was also studied by measuring an increase in the fluorescence intensity of a fluorescent lipid R_{18} incorporated into the vesicles. The increase was faster at higher concentrations of PEG. A half-time to reach the maximum fluorescence intensity decreased greatly with an increase in the PEG concentration (Table II). In the concentration range of 10–20% (w/w) of PEG 6000, the fluorescence increase should be due to dilution of R_{18} by transfer to unlabeled PC vesicles, not by fusion between the vesicles, since the completely reversible aggregation occurred at this concentration range (Figure 3).

The transfer of phospholipid molecule is also induced by dialysis of the vesicle suspension against high concentrations of impermeable PEG. Figure 8 shows transfer of 12-PC*

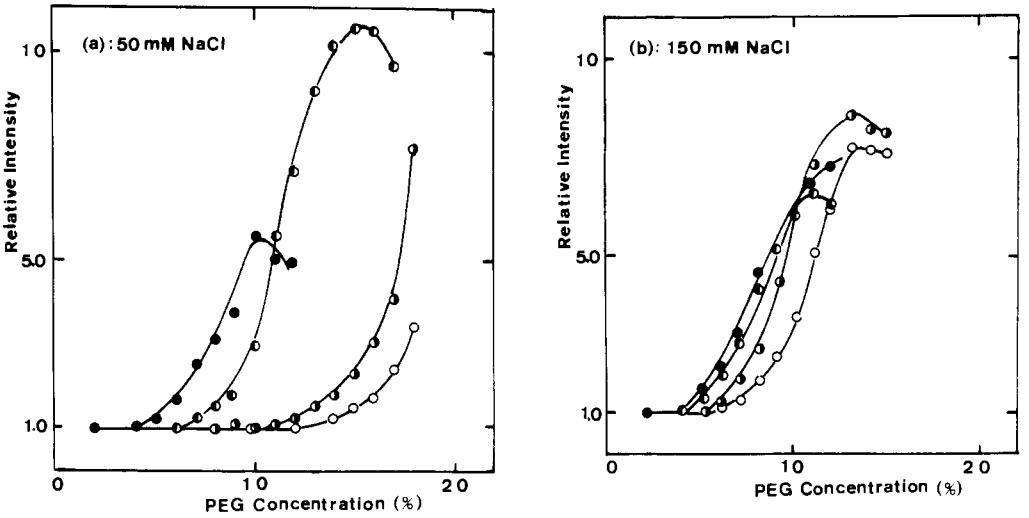

FIGURE 4: PEG-induced increase in the light scattering of egg yolk PC vesicles containing PS at various molar ratios. The vesicles were suspended in a low ionic strength medium (50 mM NaCl, 10 mM PIPES, 1 mM EDTA, pH 7.5) (a) or a high ionic strength medium (150 mM NaCl, 10 mM PIPES, 1 mM EDTA, pH 7.5) (b), and the light scattering was measured at various concentrations of PEG 6000. The molar ratio of PS to PC is 0% (●), 10% (◐), 20% (◑), and 50% (○).

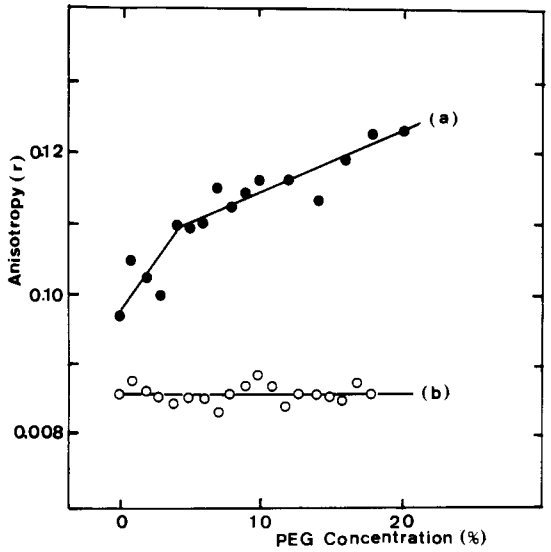

FIGURE 5: Effect of PEG on fluorescence anistropy of DPH (a) or NBD-PE (b) incorporated into egg yolk PC vesicles. The anisotropy of the individual fluorescence spectra is plotted against the concentration of PEG 6000. The molar ratio of fluorescence probe to phospholipid molecules was 1 to 500 for DPH and 1 to 100 for NBD-PE.

between the vesicles caused by dialysis against a solution containing 40% (w/w) of PEG 20 000. The transfer was initially negligible (~10 min), in contrast to that caused by the direct addition of PEG, and then gradually increased with time. The transfer immediately and completely stopped when the vesicle suspension was taken out of the dialysis bag.

Discussion

The PEG-induced decrease in the membrane fluidity and aggregation of phospholipid vesicles can be reasonably explained by the theories of osmoelastic coupling in phospholipid membranes. The phospholipid vesicles would sterically exclude high molecular weight molecules from the region adjacent to the vesicle surface (the exclusion layer) but not small molecules such as water. Therefore, the addition of a high molecular weight PEG causes an osmotic imbalance between the exclusion layer and the bulk aqueous phase, exerting an osmotic stress on the vesicles. This osmotic stress should be counterbalanced by an elastic pressure that arises from elastic strain

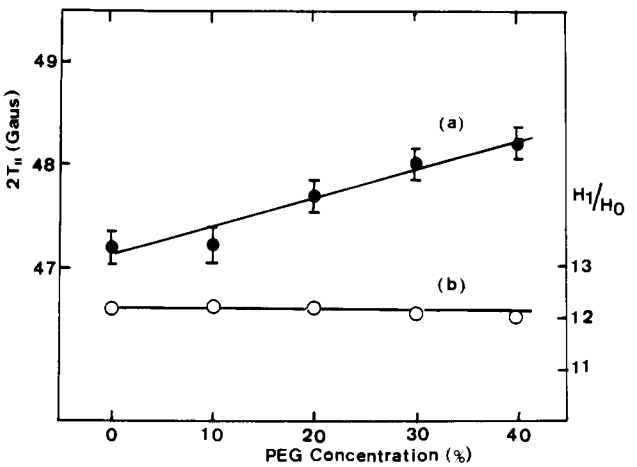

FIGURE 6: Effect of PEG on the ESR spectra of spin-labeled DMPC vesicles in the liquid-crystalline state. The maximum value of the anisotropic hyperfine splitting ($2T_{\parallel}$) for 5-SAL (a) and the ratio of the low-field peak intensity (H_1) to the middle one (H_0) for TEMPO-PC (b) are plotted against the concentration of PEG 6000. The spectra were measured at 34 °C.

of the bilayer membranes (the osmoelastic coupling). Hence the free energy of the vesicles in the dispersed state should increase with the increasing osmotic stress. In order to mitigate the strain by excluding the PEG molecules from their surroundings, the vesicles would aggregate closely to each other above a threshold intensity of the osmotic stress (the osmophobic association).

The PEG-induced decrease in the membrane fluidity detected by fluorescence anisotropy and spin-labeling suggests the osmoelastic coupling. The elastic strain caused by the osmotic stress may decrease the membrane fluidity. The observed inflection of the fluorescence anisotropy change of DPH at the onset of the vesicle aggregation might be due to increase in the light scattering. Or, more probably, the inflection should result from mitigation of the membrane strain by the osmophobic association, since an anisotropy change of NBD-PE sensing viscosity of the polar head regions was not observed at all. The PEG-induced decrease in membrane fluidity has also been observed by other investigators. Ohno et al. (1981) observed PEG-induced broadening of the proton magnetic resonance lines of DPPC membranes in the liquid-crystalline phase, whereas the resonance lines of PEG were

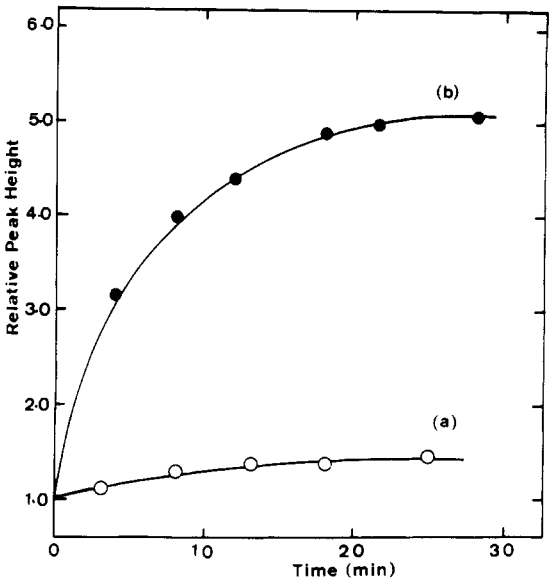

FIGURE 7: Time course of transfer of phospholipid between vesicles induced by direct addition of PEG. Egg yolk PC vesicles containing 20 mol % spin-labeled phospholipid 12-PC* were mixed with pure egg yolk PC vesicles at a ratio of 1 to 9, and the increase in the central peak height of ESR signal induced by addition of PEG 20 000 was measured at room temperature. The concentration of phospholipid in the assay mixture was 12.5 mM, and the concentration of PEG was 1% (w/w) (a) or 4% (w/w) (b).

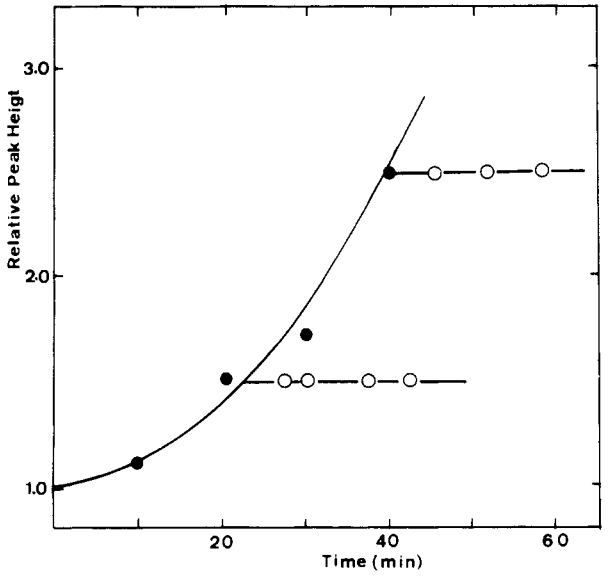

FIGURE 8: Time course of transfer of phospholipid between vesicles induced by dialysis against a high concentration of PEG. The mixture of egg yolk PC vesicles and those containing 12-PC* at the same ratio as in Figure 7 was dialyzed against a solution containing 50% (w/w) PEG 20 000 through a dialysis membrane which has a nominal molecular weight cutoff of 3500. The transfer of 12-PC* was measured in the same way as described in Figure 7. The black circles represent the time course during the dialysis, and the white ones represent the time course after taking the sample out of the dialysis bag.

not affected by DPPC vesicles. The results indicate that direct interaction between PEG molecules and membranes may not be essential for the fluidity decrease.

The PEG-induced enhancement of phospholipid transfer between vesicles which is caused without membrane fusion indicates a close contact of vesicle membranes in the PEG-induced aggregation. Such a close membrane contact can be expected from the proposed mechanism of the osmophobic association described above. The enhancement was caused not only by direct addition of PEG to the vesicle suspensions but also by dialysis of the suspension against high concentrations of PEG. In the latter, the osmotic imbalance through the dialysis membrane caused by the membrane-impermeable PEG molecules would exert an osmotic stress on the vesicle suspension. The osmotic stress does osmotic work that both removes water across the dialysis membrane and also compresses the vesicle membrane (Ito et al., 1987). Therefore, some of the osmotic energy is stored as the strain energy of the vesicle membrane, although the remainder is dissipated by the frictional interaction between the water molecules and dialysis membranes. The free energy increase due to the membrane strain causes aggregation of vesicles in the same way as that for the direct addition of PEG (osmophobic association). However, because of the dissipation of free energy due to the frictional interaction, the osmophobic association caused by dialysis is less effective and requires a higher concentration of PEG as compared with the direct addition of PEG.

The osmophobic association induced by the direct addition of cosolvent such as PEG should depend on the molecular weight of the cosolvent; larger molecular weight cosolvents would be excluded more effectively from the exclusion layer and produce larger osmotic stress. The molecular weight dependence can be quantitatively analyzed as follows. Let us assume two distinct layers, a "free diffusion layer" in which any molecule is free from steric hindrance and an "exclusion layer" near the vesicle surface from which any molecule with a radius larger than R_i is sterically excluded. When an ideal-chain molecule that possesses a radius R_0 in the free diffusion layer is packed into the exclusion layer, the resultant free energy increase can be estimated as follows (de Gennes, 1979):

$$\Delta G^\circ = -RT\Delta S \propto (R_0/R_i)^2 \propto M_w$$

where ΔG° and ΔS are the changes in the free energy and entropy and M_w is the molecular weight of the ideal-chain molecule. Assuming that PEG behaves as ideal-chain molecule, the distribution coefficient of PEG between the two layers K depends on its molecular weight M_w:

$$K = \exp(-\Delta G^\circ/RT) = \exp(-AM_w) \quad (1)$$

where A is a proportionality constant. R and T have the usual meanings. By use of the relation in eq 1, the osmotic stress on the vesicle π, which is defined as the osmolarity difference between the two layers, can be given as

$$\pi = (1 - K)C_{osm} = [1 - \exp(-AM_w)]C_{osm}$$

where C_{osm} is the osmolarity of PEG in the free diffusion layer. At the threshold osmolarity C_c at which the osmophobic association starts, the intensity of the osmotic stress should be identical, independent of the molecular weight. Therefore, the following relation holds:

$$1/C_c \propto [1 - \exp(-AM_w)] \quad (2)$$

The experimental data on the dependence of C_c on the molecular weight of PEG in Table I can be fitted well on a theoretical curve that is obtained from eq 2, by assuming a K value for PEG 20 000 of 0.15. The theoretical curve with an A value of 9.5×10^{-5}, which is given by the above value of K, is represented by the full line in Figure 2. The average molecular weight of PEG to give $K = 0.5$ is 7200 in this case, and the radius of PEG of this molecular weight value should be ~20 Å according to the estimation of Tanford (1961). These results indicate that molecules with radii larger than 20 Å have K values smaller than 0.5, thus being effectively

excluded from the exclusion layer unless they are preferentially adsorbed onto the membrane surface (de Gennes, 1979).

The increase in the threshold concentration of PEG for PS-containing vesicles shown in Figure 4 may be due to a contribution of the electrostatic repulsion between the vesicles. The electrostatic repulsive force acts effectively only on the vesicles close enough to each other. Hence it should increase preferentially the free energy of vesicles in the aggregated state, so that it should require an additional intensity of the osmotic stress to cause the osmophobic association. According to the theoretical analysis of the osmotic response of phospholipid vesicles (Ito, Yamazaki, and Ohnishi, submitted for publication), there holds the following relation between the interbilayer repulsive force and the osmotic stress that counterbalances the force:

$$\pi_\sigma = (1/\phi_w)P_R \quad (3)$$

where π_σ, P_R, and ϕ_w are the osmotic stress, interbilayer repulsive force acting on the unit surface area of the aggregated vesicles, and volume fraction of water in the intervesicular area in the aggregation, respectively. The increase in the threshold concentration for PS-containing vesicles should correspond to the increase in the threshold osmotic stress, which could be represented by eq 3. P_R is the electrostatic repulsive force resulting from the negative surface charge of PS-containing vesicles, in this case (Overbeek, 1953). It should increase with the increase in the surface charge density (PS content) and decrease with the increase in the ionic strength in the medium, as actually observed (Figure 4).

Generally, dispersion systems of colloidal particles can be classified into two categories, i.e., reversible (hydrophilic) and irreversible (hydrophobic) systems. In the former, the dispersed state is thermodynamically stable. In the latter, the aggregated state is thermodynamically stable and particles that have been once aggregated cannot be disaggregated reversibly. Hitherto, phospholipid vesicles have been regarded as hydrophobic colloidal particles and the stability of the dispersed state has been considered as a kinetically stable state owing to a high activation energy of the aggregation process, as analyzed for the systems of metal colloids (Overbeek, 1952). The hypothetical "dehydration force" as well as the electrostatic force has been assumed to cause the high activation energy (Lis et al., 1982). However, the results in the present report strongly suggest that dispersion of the vesicles is a thermodynamically stable state. The reversible transition to the aggregated state induced by the osmotic stress and the observed phospholipid exchange between vesicles in the aggregation indicate that the osmophobic association of the vesicles, in which the vesicle membranes closely contact each other, should be another thermodynamically stable state. The close membrane contact may correspond to the tight junction of plasma membrane observed in cell–cell adhesions.

Registry No. PEG, 25322-68-3.

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