Deformation and Instability in Membrane Structure of Phospholipid Vesicles Caused by Osmophobic Association: Mechanical Stress Model for the Mechanism of Poly(ethylene glycol)-Induced Membrane Fusion†

Masahito Yamazaki‡ and Tadanao Ito*
Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan
Received May 12, 1989; Revised Manuscript Received October 5, 1989

ABSTRACT: The mechanism of poly(ethylene glycol)-induced fusion of phospholipid vesicles was studied based on the "osmophobic association" theory which was recently proposed both theoretically [Ito, T., Yamazaki, M., & Ohnishi, S. (1989) *Biochemistry 28*, 5626–5630] and also experimentally [Yamazaki, M., Ohnishi, S., & Ito, T. (1989) *Biochemistry 28*, 3710–3715]. Osmophobic association and fusion were detected by measuring the light scattering of the vesicle suspension; the former was detected from the increase in light scattering induced by the addition of PEG, and the latter was from the irreversibility of the increase in light scattering. Threshold concentrations of PEG were required not only for osmophobic association but also for fusion. The threshold concentration for fusion depended on the molecular weight of PEG and also on the electrostatic repulsive interaction betwen phospholipid vesicles, which was manipulated by the use of vesicles with negative surface charge; increasing the molecular weight of PEG lowered the threshold concentration, and increasing the electrostatic repulsive interaction raised it. In addition, a transient leakage of internal contents from the vesicles was observed at the concentration that caused fusion. When the surface charge of the vesicle was varied, the threshold for fusion coincided with that for osmophobic association, provided that the latter exceeded 22 wt % of PEG 6000. However, when the threshold for osmophobic association was less than 22 wt %, the threshold for fusion remained ~22 wt %, irrespective of the difference in the threshold for osmophobic association. Electron microphotographs of quick-frozen replicas of egg yolk phosphatidylcholine vesicles showed that the vesicles in the aggregate caused by PEG-induced osmophobic association were deformed to increase their area of contact with the adjacent vesicles. According to the analysis based on the osmophobic association theory, the mechanical force (f) that causes the deformation of the vesicle (deformation force) is counterbalanced by the thermodynamic force due to osmophobic association, increasing with increased concentrations of PEG, but it is little affected by the electrostatic repulsive interaction between the vesicles. On the basis of the results described above, a "mechanical stress model" is proposed for the mechanism of PEG-induced fusion. Membranes that are tightly associated by osmophobic association are mechanically strained by the deformation force f. Consequently, the membrane structure becomes unstable at increased concentrations of PEG, and above a critical concentration, ~22 wt % of PEG 6000, destruction of the bilayer structure into a leaky membrane structure may cause fusion.

High molecular weight poly(ethylene glycol) (PEG)[1] is a water-soluble fusogen which has been used frequently to fuse somatic cells (Davidson & Gelald, 1977; Lucy, 1977). It also causes aggregation of phospholipid vesicles and, at relatively high concentrations, induces fusion (Tilcock & Fisher, 1982; Boni et al., 1981; Saez et al., 1982; Boni & Hui, 1987). To understand the mechanism of PEG-induced membrane fusion, it is essential to elucidate the effects of PEG on the stability of phospholipid bilayer membranes.

It has been emphasized that direct binding of PEG may perturb the structure of phospholipid bilayer membranes. Boni et al. (1984) suggested a possibility that "rigididation" of the phospholipid molecules by the direct binding of PEG may induce defects in the bilayer which causes membrane fusion. However, the concentration of PEG needed to cause membrane fusion was much higher than the estimated concentration for the saturated binding of PEG. Saez et al. (1982) observed that PEG 1000 caused leakage of internal contents from egg yolk PC vesicles. From the results, they claimed that a detergent-like action of PEG may cause membrane fusion.

On the other hand, MacDonald (1985) has shown that in a suspension of phospholipid vesicle separated from a solution of higher osmolarity by a dialysis membrane, exchange of the phospholipid molecules between the membranes and fusion of the vesicles occurred. These results strongly suggest that membrane fusion caused by the addition of PEG does not necessarily require any direct interaction of PEG with the vesicles.

Recently, we proposed the novel concepts of "osmoelastic coupling" and "osmophobic association" (Ito et al., 1989; Yamazaki et al., 1989). On the basis of these concepts, the mechanism of the PEG-induced aggregation of phospholipid vesicles is explained as follows. In a suspension of phospholipid vesicles containing a high molecular weight PEG, the PEG molecules are preferentially excluded from the region adjacent to the vesicle surface (exclusion layer), but not small molecules such as water or inorganic ions. Such exclusion induces osmotic stress onto the vesicles. Therefore, to create an elastic pressure which counterbalances the osmotic stress, the membrane of the vesicle is compressed (osmoelastic coupling).

† This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan.
* To whom correspondence should be addressed.
‡ Present address: Department of Physics, Faculty of Science, Shizuoka University, Shizuoka 422, Japan.

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); PEG, poly(ethylene glycol); PC, phosphatidylcholine; PS, phosphatidylserine.

0006-2960/90/0429-1309$02.50/0

Consequently, the free energy of the vesicle in the dispersed state is increased, and above a critical concentration of PEG, the vesicle aggregates tightly with each other, in order to reduce the free energy increase (osmophobic association).

In this report, we show that PEG-induced osmophobic association may cause fusion of phospholipid vesicles. The osmophobic association can be stabilized by the tight aggregation of the vesicles that excludes the solvent from their area of contact with the adjacent vesicles. Therefore, to increase the contact area as much as possible, the vesicles are remarkably deformed, followed by a concomitant instability in the membrane structure. The instability proceeds with an increase in concentration of PEG, so that above a critical concentration of PEG, membrane fusion is induced.

Materials and Methods

Phosphatidylcholine from egg yolk (egg yolk PC) and phosphatidylserine from beef brain white matter (PS) were prepared according to the methods of Singleton et al. (1965) and Sanders et al. (1967). Phosphatidic acid prepared from egg yolk PC was purchased from Sigma Chemical Co. 8-Aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS) and *p*-xylylenebis(pyridinium bromide) (DPX) were purchased from Molecular Probes Inc. PEG 6000 (average molecular weight 7500) and PEG 1000 (average molecular weight 1000) were purchased from Wako Chemical Co.

Unilamellar phospholipid vesicles were prepared by sonicating the phospholipid suspension with a tip-type sonicator. PEG-induced aggregation of the sonicated vesicles was detected from an increase in light scattering of the vesicle suspension induced by the addition of PEG. The irreversibility of aggregate formation was detected from the irreversible increase in the light scattering after dilution of the suspension 20-fold by PEG-free solution (Yamazaki et al., 1989).

The leakage of internal contents from phospholipid vesicles was assayed according to the method of Ellens et al. (1984). Briefly, phospholipid vesicles containing fluorescence probe ANTS and its quencher DPX were prepared in solution containing 12.5 mM ANTS, 45 mM DPX, 53.8 mM NaCl, and 10 mM PIPES (pH 7.5) (A buffer), or in solution containing 2.5 mM ANTS, 9.0 mM DPX, and 5 mM PIPES (B buffer). To remove the untrapped ANTS or DPX, the vesicles prepared in A buffer were eluted on a Sephadex G-75 column with 10 mM PIPES-buffered solution (pH 7.5, PIPES buffer) containing 140 mM NaCl and the ones prepared in B buffer with PIPES buffer containing 10 mM NaCl. The vesicle suspension eluted with PIPES buffer containing 10 mM NaCl was diluted 2-fold with water. For the assay of the leakage of ANTS, the vesicle suspension was diluted 5-fold with PIPES buffer containing 140 mM NaCl (H buffer) or with NaCl-omitted PIPES buffer (L buffer), which contained an appropriate concentration of PEG 6000. The rate of leakage of ANTS was estimated after the correction of the fluorescence enhancement of ANTS by PEG. EDTA was added to the solutions used in the case of the vesicles containing the anionic lipids (final concentration 1 mM).

The quick-frozen replicas for the electron microscope were prepared as follows: 10 mg/mL sonicated egg yolk PC vesicles was eluted on a Sephadex 6B column with H buffer. The eluted sample was suspended in an equivolume of H buffer containing 40 wt % PEG 6000. Then, the small drops of the suspension were quick-frozed by slamming against a helium-cooled copper block according to Heuser and Salpeter (1979). The quick-frozen samples were freeze-fractured at −135 °C and etched at −100 °C for 2 min in the vacuum below 2×10^{-6} torr, using a Balzer's BAF 300 freeze-fracture device.

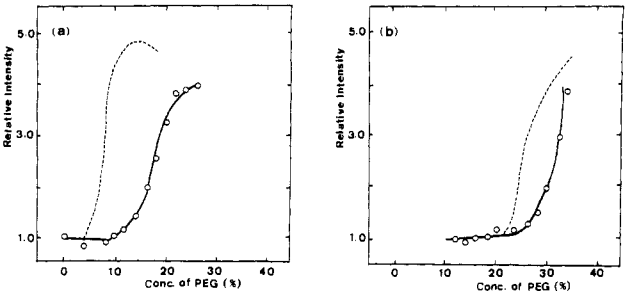

FIGURE 1: PEG-induced increase (a) and irreversibility (b) in light scattering of the egg yolk PC vesicle suspension. The sonicated egg yolk PC vesicles were suspended in PIPES-buffered solution of high ionic strength (10 mM PIPES–140 mM NaCl, pH 7.5; H buffer) containing PEG 1000. The increase in light scattering induced by PEG 1000 and its irreversibility were detected by measuring the light scattering of the suspension at the individual concentrations of PEG 1000 [(O) in (a)] and the light scattering after 20-fold dilution of the suspensions with PEG-free H buffer [(O) in (b)], respectively. The dashed lines in (a) and in (b) are the results of these experiments for PEG 6000 obtained by Yamazaki et al. (1989).

Replicas were made by rotary shadowing with an platinum/carbon electron beam gun mounted at 25° relative to the fractured frozen sample. The replicas were photographed in a JEM 200CX electron microscope operated at 200 kV. The electron microphotographs were photographically reversed before demonstration.

Results

PEG-Induced Increase and Irreversibility in Light Scattering of Phospholipid Vesicle Suspensions. Addition of high molecular weight PEGs caused an increase in light scattering of sonicated phospholipid vesicles which results from formation of aggregation of the vesicles (Figure 1a). As proposed by Yamazaki et al. (1989), the aggregation should be an osmophobic association; osmotic stress arising from preferential exclusion of PEG from the region adjacent to the vesicle surface causes the aggregation of the vesicles by increasing preferentially the free energy in the dispersed state. The minimum concentration to cause osmophobic association (threshold concentration for osmophobic association) was smaller for PEG with a larger molecular weight (Figure 1a) (Yamazaki et al., 1989). At relatively high concentrations of PEG, irreversibility of the increase in light scattering was observed; i.e., the PEG-induced increase in light scattering was not cleared away after dilution of the suspension with a PEG-free medium (Figure 1b). The irreversibility should result from formation of large vesicles or multilayer vesicles which follows fusion of the vesicle membranes as indicated by others (Saez et al., 1982; Boni et al., 1984). The concentration of PEG at which the irreversibility emerged (threshold concentration for irreversibility) depended on its molecular weight like the threshold concentration for osmophobic association. For example, the threshold concentrations for irreversibility were 22 wt % for PEG 6000 and 28 wt % for PEG 1000 in the case of egg yolk PC vesicles.

Anionic lipid such as phosphatidylserine (PS) contained in the vesicle membrane increased not only the threshold concentration for osmophobic association but also that for irreversibility. Such effects of anionic lipid were remarkably dependent on the ionic strength of the suspension medium. For example, in the case of egg yolk PC–50 mol % PS vesicles, the threshold concentrations of PEG 6000 for osmophobic association and for irreversibility were 9 and 22 wt %, respectively, in a solution containing 140 mM NaCl, whereas both of them increased to 28 wt % in the absence of NaCl (Figure 2). On the other hand, either of them does not depend

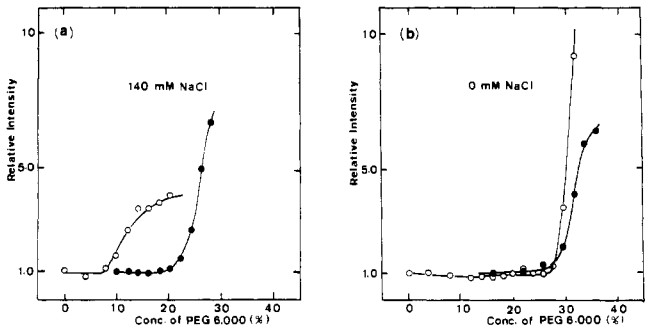

FIGURE 2: PEG-induced increase and irreversibility in light scattering of egg yolk PC–50 mol % PS vesicle suspensions. The increase in light scattering (○) induced by PEG 6000 and its irreversibility (●) were detected for the sonicated egg yolk PC–50 mol % PS vesicles suspended in H buffer (a) or in NaCl-omitted PIPES-buffered solution (L buffer) (b) by the same way as described in Figure 1.

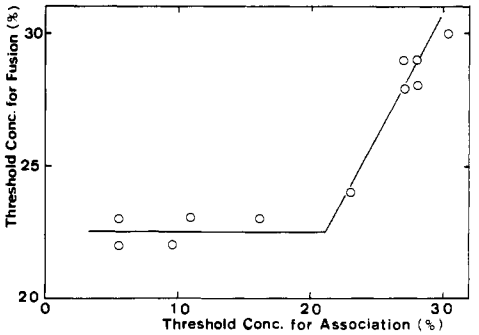

FIGURE 3: Relationship of threshold concentraions of PEG 6000 for irreversibility with those for osmophobic association. The threshold concentrations for irreversibility in Table I are plotted as a function of those for osmophobic association.

Table I: Threshold Concentrations of PEG 6000 for Osmophobic Association, for Irreversibility in Light Scattering, and for Leakage of Internal Contents[a]

PS content (mol %)	[NaCl] (mM)	[PEG] for association (%)	[PEG] for irrevers-ibility (%)	[PEG] for leakage (%)
0	140	5.5	22	22–23
0	40	5.5	23	nd[b]
5	0	10.5	23	nd
20	0	27	29	nd
50	0	28	29	nd
50	1	30	30	30–32
50	10	23	24	nd
50	40	16	23	nd
50	140	9.5	22	22

[a] The sonicated vesicles composed of egg yolk PC and PS at various molar ratios were suspended in PIPES-buffered solutions (10 mM PIPES, pH 7.5, for PC vesicles or 10 mM PIPES–1 mM EDTA, pH 7.5, for PC–PS vesicles) containing various concentrations of NaCl, and threshold concentrations of PEG 6000 for osmophobic association, for irreversibility, and for leakage were determined as described in the text. [b] Not determined.

on the ionic strength in the case of egg yolk PC vesicles (Table I). In addition, two different anionic lipids, PS and phosphatidic acid (PA), had quite the same effects. Therefore, the above-mentioned effects should be attributed solely to the negative surface charge of the vesicles given by the anionic lipid. The threshold concentrations for osmophobic association and also for irreversibility of egg yolk PC–X mol % (X = 0–50) PS vesicles are summarized in Table I, and the threshold concentrations for irreversibility are plotted as a function of those for osmophobic association in Figure 3.

Leakage of Internal Contents from Phospholipid Vesicles. Leakage of internal contents from phospholipid vesicles induced by the addition of PEG was assayed by use of a mixture

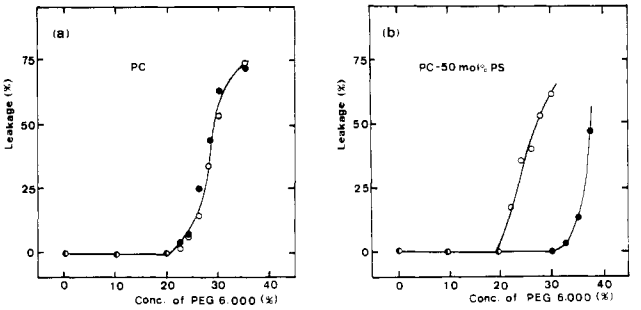

FIGURE 4: PEG-induced leakage of internal contents from egg yolk PC and egg yolk PC–50 mol % PS vesicles. The sonicated vesicles of egg yolk PC (a) or egg yolk PC–50 mol % PS (b) containing a fluorescence probe, ANTS, and its quencher, DPX, were suspended in H buffer (○) or in L buffer (●) containing PEG 6000 at various concentrations. Leakage of the internal contents at the individual concentrations of the PEG was assayed from the increase in fluorescence intensity of ANTS, according to the procedure described under Materials and Methods.

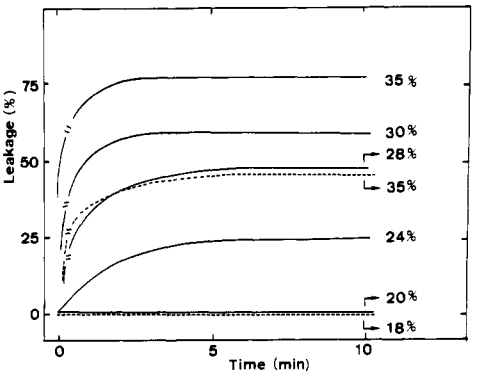

FIGURE 5: Time course of PEG-induced leakage of internal contents from sonicated vesicles and multilayer liposomes of egg yolk PC. The time course of leakage of the internal contents from the sonicated egg yolk PC vesicle (solid line) or the multilayer liposome (dashed line) was measured in H buffer containing PEG 6000 at the concentrations denoted in the figure.

of the fluorescent probe ANTS and its quencher DPX as the internal contents (Figures 4 and 5). For egg yolk PC vesicles, the leakage was observed at concentrations of PEG 6000 ≥22 wt % (Figure 4). The extent of leakage reached a stationary level within 10 min, and the stationary value increased with an increase in concentration of PEG (Figure 5). The leakage from egg yolk PC vesicles was independent of the ionic strength of the suspension medium (Figure 4a). On the other hand, the leakage from the vesicles containing anionic lipid was dependent on ionic strength; a higher concentration of PEG was required to cause leakage in medium of low ionic strength than that of high ionic strength (Figure 4b). In a solution containing 140 mM NaCl, the minimum concentration of PEG for leakage (threshold concentration for leakage) from egg yolk PC–50 mol % PS vesicles was as low as that from pure egg yolk PC vesicles [(○) in Figure 4a,b]. Moreover, the threshold concentrations for leakage were almost the same as those for irreversibility (Table I). The PEG-induced leakage of internal contents was also observed in multilayer liposomes, though the extent was a little bit smaller (dashed lines in Figure 5).

Deformation of Phospholipid Vesicles Caused by PEG-Induced Osmophobic Association. Using negative-stain electron microscopy, Saez et al. (1982) showed that egg yolk PC vesicles aggregated by the addtion of PEG were largely deformed. Such deformation of egg yolk PC vesicles were also observed using quick-frozen replica electron microscopy. The vesicle in the aggregate increases the area of contact with the adjacent vesicle as a consequence of the deformation, as in-

FIGURE 6: Electron micrograph of a quick-frozen replica of egg yolk PC vesicles in the presence of 20 wt % PEG 6000. The replica of the sonicated egg yolk PC vesicles suspended in H buffer containing 20 wt % PEG 6000 was prepared according to the procedure described under Materials and Methods. The electron micrograph of the replica was demonstrated after it was photographically reversed. The arrow indicates the increase in the contact area caused by deformation of the vesicles as discussed in the text. Scale bar, 50 nm.

dicated by the arrow in Figure 6. The mechanism to cause such a large deformation may be as follows. The vesicles in the aggregate may tend to make the contact area as large as possible, because the osmophobic associations are stabilized by the membrane contact that reduces the effect of the osmotic stress by excluding the solvent from the contact area (Yamazaki et al., 1989). Consequently, the deformation may proceed to the state at which the decrease in the free energy by the osmophobic association is balanced with the increase by the deformation.

To analyze it quantitatively, we shall consider the increase in the free energy of the vesicle (dF_a) concomitant with an increase in the contact area (dS_a). Two terms should contribute to dF_a, i.e., dF_o and dF_d; the former represents the increase in the free energy due to osmophobic association and the latter is due to deformation of the vesicles.

In the case of neutral phospholipid vesicles, in which the electrostatic interaction due to the surface charge can be neglected, dF_o is represented as (Ito et al., 1989; Yamazaki et al., 1989)

$$dF_o = -[(1/S_t)F_c dS_a + (1/S_t)F_{os} dS_a]$$

where F_c represents the free energy due to the contact interaction between the adjacent membranes such as van der Waals interaction, using our sign convention for stabilization of the contact (contact interaction energy), F_{os} represents the free energy due to the osmoelastic coupling in the vesicle subject to osmotic stress (osmotic stress energy), and S_t is the total surface area of one vesicle. The first term in brackets on the right-hand side corresponds to the increase in the contact interaction energy, and the second term corresponds to the decrease in the osmotic stress energy *by osmophobic association*. As analyzed by Ito et al. (1987) and Suzuki et al. (1989), F_{os} should be proportional to the square of the osmotic stress, C_{osm}

$$F_{os} = AC_{osm}^2$$

where A is the proportionality constant. In an osmotically ideal solution, C_{osm} can be represented as

$$C_{osm} = RTKC$$

where C is the concentration of PEG, K is the exclusion coefficient of PEG from the exclusion layer adjacent to the vesicle surface, R is the gas constant, and T is the absolute temperature. By use of the partition coefficient of PEG between the exclusion layer and the bulk phase (K'), K is represented by $1 - K'$ (Yamazaki et al., 1989). In later discussions, we shall assume the system as osmotically ideal for simplicity, since the essential points of our arguments are not affected by the assumption.

From the results discussed above, the increase in the free energy of the vesicle in the aggregate dF_a is represented as

$$dF_a = dF_o + dF_d = -(1/S_t)[F_c + A(RTK)^2C^2]dS_a + dF_d \quad (1)$$

From the condition for the formation of thermodynamically stable aggregation, the following relation can be derived. Since

$$\partial F_a/\partial S_a = 0$$

then

$$f \equiv \partial F_d/\partial S_a = (1/S_t)[A(RTK)^2C^2 + F_c] \quad (2)$$

Equation 2 shows that the mechanical force f that causes the deformation of the vesicle (deformation force) is counteracted with the thermodynamic force arising from osmophobic association *in the thermodynamically stable aggregate*. The intensity of f increases with increased concentrations of PEG. Therefore, the vesicles in the aggregate are deformed more and more with the increase in concentration of PEG.

In the case of negatively charged vesicles, we should take into consideration the electrostatic repulsive interaction between the vesicles. To estimate the effects of the interaction on the osmophobic association and the deformation force, we shall introduce the parameters

$$P_R \equiv dF_{elec}/dr$$

$$\delta P_R \equiv dF_{elec}/dS_a = P_R dr/dS_a$$

where F_{elec} is the free energy due to the electrostatic interaction and r is the distance between the vesicle surfaces. R_R corresponds to a repulsive force due to the electrostatic interaction between the vesicles. As shown by Ito et al. (1989) and Yamazaki et al. (1989), P_R increases the intensity of the osmotic stress needed to cause osmophobic association. δP_R corresponds to the increase in the electrostatic repulsive interaction caused by the deformation. δP_R should contribute to the change in the free energy of the vesicle *in the aggregate* dF_a, adding a term of $\delta P_R dS_a$ to eq 1. However, δP_R may be negligibly small, since the change in r concomitant with the change in S_a should be so small that $dr/dS_a \approx 0$. Therefore, the surface charge should hardly affect the deformation force f.

The results of the above analysis are schematically represented in Figure 7. The free energy of the vesicle F_a in the aggregate has the minimum value at $S_a = (S_a)_s$, at which *the thermodynamically stable aggregate* is formed. The minimum value of the free energy depends on the electrostatic repulsive force P_R, increasing with an increase in P_R (compare A with B in Figure 7). On the other hand, the deformation force f acting on the vesicle is independent of the surface charge of the vesicle, although the force increases with increased concentrations of PEG according to eq 2 as shown in the insert of Figure 7.

Discussion

In this report, we analyzed the deformation of phospholipid vesicles caused by the addition of PEG according to the osmophobic association theory recently proposed by Ito et al. (1989) and Yamazaki et al. (1989). On the basis of the results

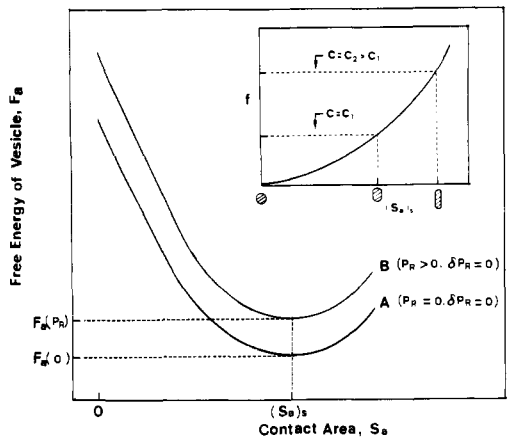

FIGURE 7: Schematic representation of the free energy of the phospholipid vesicle in the PEG-induced aggregate F_a as a function of the contact area S_a. $(S_a)_s$ represents the contact area in the thermodynamically stable aggregate. P_R ($=dF_{elec}/dr$) and δP_R ($= dF_{elec}/dS_a$) are the parameters of the electrostatic repulsive interaction, where F_{elec} and r are the electrostatic repulsive energy and the distance between the two membranes adjacent to each other, respectively, $F_a(0)$ and $F_a(P_R)$ are the minimum value of the free energy represented by A, where $P_R = 0$ and $\delta P_R = 0$, and that represented by B, where $P_R > 0$ and $\delta P_R = 0$, respectively. The relationship of the deformation force f to $(S_a)_s$ and also the deformation of the vesicle at two concentrations of PEG, C_1 and C_2, is shown schematically in the insert. See the text for details.

of the present analysis, we proposed the following "mechanical stress model" for the mechanism of PEG-induced fusion of phospholipid vesicles.

As is well recognized, fusion of bilayer membranes should be preceded by tight association of the membranes. The osmophobic association of phospholipid vesicles induced by the addition of PEG can make such tight association of the bilayer membranes (Yamazaki et al., 1989). Induction of some instability in the membrane structure should be necessary for the fusion to proceed. The deformation force f that acts on the vesicle in the aggregate due to the PEG-induced osmophobic association can induce such instability. As represented by eq 2, f is balanced with the thermodynamic force arising from the osmophobic association, increasing with increased concentrations of PEG. Hence, the instability in the membrane structure induced by f becomes larger with the increase in concentration of PEG. Consequently, above a critical concentration of PEG, the membrane fusion may occur at the area tightly associated by osmophobic association. This putative mechanism of membrane fusion can be called the "mechanical stress model".

The PEG-induced leakage of the internal contents from the vesicle might be due to the breakdown of the vesicular structure following the fusion. However, the leakage was also observed in the multilayer liposome (Figure 5). In this case, the internal contents have to leak out through the inner membranes which should not participate in the fusion. Therefore, the breakdown of the vesicular structure should not be the cause of the leakage.

Alternatively, the observed leakage should be due to destruction of the bilayer structure into a leaky membrane structure, which may be caused by the deformation force as discussed above. The results in Figure 4 and Table I show that the threshold concentrations for leakage (the minimum concentrations to cause the leakage) are almost the same as those for fusion detected by the irreversibility. It suggests that such destruction of the bilayer structure may bring about membrane fusion.

An interesting result was obtained in the relation between the threshold concentration of PEG for fusion and that for osmophobic association (Figure 3). When the threshold concentration for osmophobic association did not exceed a critical concentration, 22 wt % of PEG 6000, the threshold concentration for fusion remained 22–23 wt %, irrespective of the difference in the threshold for osmophobic association. However, when the threshold concentration for osmophobic association exceeded 22 wt %, the threshold for fusion coincided with the threshold for osmophobic association.

The result mentioned above can be explained reasonably, based on the "mechanical stress model". As already discussed, the electrostatic interaction due to the surface charge of the vesicles does not affect so much the deformation force f that gives the mechanical strain to the vesicle in the aggregate, whereas the interaction increases the threshold concentration for osmophobic association (Figure 7). Therefore, the intensity of f needed to cause fusion of the vesicles should not correlate directly with the threshold concentration for osmophobic association; 22 wt % PEG 6000 should be the concentration to yield the minimum intensity of f for fusion. Hence, fusion does not occur until 22 wt % in the vesicle suspension where the threshold concentration for osmophobic association is less than 22 wt %. On the other hand, in the vesicle suspension where the threshold concentration for osmophobic association is more than 22 wt %, osmophobic association and fusion occur simultaneously, because f should exceed the critical intensity at any concentration that causes the aggregation, in this case.

The PEG-induced fusion depends on the molecular weight of PEG. Larger molecular weight PEG is more effective (Figure 1). The dependence may be explained by the "mechanical stress model" as follows. The osmotic stress is expected to be larger for larger molecular weight PEG, since larger molecules should be excluded more effectively from the exclusion layer adjacent to the membrane surface (Yamazaki et al., 1989). Therefore, the deformation force f increases with the increase in molecular weight of PEG. Consequently, PEG with a larger molecular weight should be able to induce the membrane instability and cause fusion at a lower concentration (Figure 1).

Many factors have been considered to explain the mechanism of PEG-induced membrane fusion: binding of PEG to phoshpolipid molecules in the membrane (Boni et al., 1984); detergent-like action of PEG (Saez et al., 1982); PEG-induced change of aqueous phase properties (Herrmann et al., 1983) or so-called dehydration effects of PEG (MacDonald, 1985); etc. On the other hand, the mechanism proposed here, based on the osmophobic association theory, does not require any special assumption. It can also explain the interesting observation by MacDonald (1985) that dialysis of a suspension of phospholipid vesicle against a solution of higher osmolarity causes exchange of the phospholipid molecules and also fusion of the vesicles like the direct addition of PEG. In this case, it is osmotic stress across the dialysis membrane that acts upon the lipid suspension. The osmotic stress does osmotic work that both removes water across the dialysis membrane and also stresses the bilayer membrane by osmoelastic coupling (Ito et al., 1987; Yamazaki et al., 1989). Consequently, osmophobic association and fusion may be caused in the same way as they are caused by the direct addition of PEG.

ACKNOWLEDGMENTS

We thank Professor Shun-ichi Ohnisihi of Kyoto University for his constant encouragement during this study and also Yoko Ikeuchi of Tokyo Metropolitan Institute for Neuroscience for preparing the quick-frozen replicas and taking their electron micrographs.

REFERENCES

Boni, L. T., & Hui, S. W. (1987) in *Cell Fusion* (Sowers, A. E., Ed.) pp 301–330, Plenum Press, New York.

Boni, L. T., Alderfer, J. L., & Hui, S. W. (1981) *J. Membr. Biol. 62*, 65–70.

Boni, L. T., Hah, J. S., Hui, S. W., Mukherjee, P., Ho, J. T., & Jung, C. Y. (1984) *Biochim. Biophys. Acta 775*, 409–418.

Davidson, R. L., & Gerald, P. S. (1976) *Somatic Cell Genet. 2*, 165–176.

Ellens, H., Bentz, J., & Szoka, F. C. (1984) *Biochemistry 23*, 1532–1538.

Herrman, A., Pratsch, L., Arnold, K., & Lassmann, G. (1983) *Biochim. Biophys. Acta 733*, 87–94.

Heuser, J. E., & Salpeter, S. R. (1979) *J. Cell Biol. 82*, 150–173.

Ito, T., Zaner, K. S., & Stossel, T. P. (1987) *Biophys. J. 51*, 745–753.

Ito, T., Yamazaki, M., & Ohnishi, S. (1989) *Biochemistry 28*, 5626–5630.

Lucy, J. A. (1977) in *Structure of Biological Membranes* (Abrahamson, S., & Pascher, I., Eds.) Plenum Press, New York.

MacDonald, R. I. (1985) *Biochemistry 24*, 4058–4066.

Saez, R., Alonso, A., Villena, A., & Goni, F. M. (1982) *FEBS Lett. 137*, 323–326.

Sanders, H. (1967) *Biochim. Biophys. Acta 144*, 485–487.

Singleton, W. S., Gray, M. S., Brown, M. L., & White, J. L. (1965) *J. Am. Oil Chem. Soc. 42*, 53–56.

Suzuki, A., Yamazaki, M., & Ito, T. (1989) *Biochemistry 28*, 6513–6518.

Tilcock, C. P. S., & Fisher, D. (1982) *Biochim. Biophys. Acta 688*, 645–652.

Yamazaki, M., Ohnishi, S., & Ito, T. (1989) *Biochemistry 28*, 3710–3715.