Biochimica et Biophysica Acta, 775 (1984) 409–418
Elsevier

BBA 72173

AGGREGATION AND FUSION OF UNILAMELLAR VESICLES BY POLY(ETHYLENE GLYCOL)

L.T. BONI [a,*], J.S. HAH [a], S.W. HUI [a,**], P. MUKHERJEE [b], J.T. HO [b] and C.Y. JUNG [c]

[a] *Biophysics Department, Roswell Park Memorial Institute, Buffalo, NY 14263,* [b] *Physics Department, State University of New York at Buffalo, Buffalo, NY 14260 and* [c] *Biophysics Laboratory, Veterans Administration Medical Center and Biophysical Science Department, State University of New York at Buffalo, Buffalo, NY 14215 (U.S.A.)*

(Received December 14th, 1983)
(Revised manuscript received April 10th, 1984)

Key words: Poly(ethylene glycol); Vesicle aggregation; Membrane fusion

Various aspects of the interaction between the fusogen, poly(ethylene glycol) and phospholipids were examined. The aggregation and fusion of small unilamellar vesicles of egg phosphatidylcholine (PC), bovine brain phosphatidylserine (PS) and dimyristoylphosphatidylcholine (DMPC) were studied by dynamic light scattering, electron microscopy and NMR. The fusion efficiency of Dextran, glycerol, sucrose and poly(ethylene glycol) of different molecular weights were compared. Lower molecular weight poly(ethylene glycol) are less efficient with respect to both aggregation and fusion. The purity of poly(ethylene glycol) does not affect its fusion efficiency. Dehydrating agents, such as Dextran, glycerol and sucrose, do not induce fusion. ^{31}P-NMR results revealed a restriction in the phospholipid motion by poly(ethylene glycol) greater than that by glycerol and Dextran of similar viscosity and dehydrating capacity. This may be associated with the binding of poly(ethylene glycol) to egg PC, with a binding capacity of 1 mol of poly(ethylene glycol) to 12 mol of lipid. Fusion is greatly enhanced below the phase transition for DMPC, with extensive fusion occurring below 6% poly(ethylene glycol). Fusion of PS small unilamellar vesicles depends critically on the presence of cations. Large unilamellar vesicles were found to fuse less readily than small unilamellar vesicles. The results suggest that defects in the bilayer plays an important role in membrane fusion, and the 'rigidization' of the phospholipid molecules facilitates fusion possibly through the creation of defects along domain boundaries. Vesicle aggregation caused by dehydration and surface charge neutralization is a necessary but not a sufficient condition for fusion.

Introduction

Membrane fusion is an important event both at the cellular and subcellular levels. Studying the details of chemically induced fusion is a means of approaching the ultrastructural changes and molecular mechanisms involved in cell fusion [1]. Understanding the molecular mechanism of poly(ethylene glycol)-induced fusion may lead to methods of improving the fusion efficiency, and permit hybridization and other modifications in cellular properties to be more accurately engineered.

During the process of poly(ethylene glycol)-induced fusion between cells, intramembrane-particle-free regions in cell plasma membranes are created and are thought to be sites of cell fusion [2–5]. Since these sites are likely to represent ex-

* Present address: Department of Pharmacology, Harvard Medical School, Boston, MA 02115, U.S.A.
** To whom correspondence should be addressed.

Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine.

posed lipid bilayers, the study of pure lipid systems appears to be a necessary first approach to understanding poly(ethylene glycol)-induced fusion. Aggregation and fusion of small unilamellar vesicles in poly(ethylene glycol) has been shown [6,7], and the threshold concentrations for vesicle aggregation and fusion has been measured. This present study aims at defining various factors that influence the fusion efficiency of poly(ethylene glycol). The factors examined include dehydration, binding, and the structural phase, surface charge and the size of the lipid vesicles. The result suggests that the fusogenic activity of poly(ethylene glycol) is based upon a multitude of factors.

Materials and Methods

Materials. Hen egg phosphatidylcholine (egg PC) and bovine brain phosphatidylserine (bovine PS) were purified by the method of Papahadjopoulos and Miller [8]. All other phospholipids used were purchased from Avanti Polar-Lipids (Birmingham, AL). All lipids were stored in chloroform and were shown to be pure by thin-layer chromatography.

Poly(ethylene glycol) (Carbowax) of average M_r 6000 was purchased from Fisher Scientific (Fair Lawn, NJ). All other molecular weight poly(ethylene glycol) was obtained from Sigma Chemical Co. Dextran, of M_r 200–300 kDa, was obtained from Nutritional Biochemicals Co. (Cleveland, OH). Tritiated poly(ethylene glycol) were purchased from New England Nuclear (Boston, MA).

In experiments investigating the effect of impurities, the poly(ethylene glycol) was purified according to the method of Honda et al. [9] by dissolving poly(ethylene glycol) in chloroform followed by reprecipitation into diethyl ether. Further purification (to remove water-soluble impurities) was accomplished by dialysing 5% poly(ethylene glycol) (w/v) against distilled water for 3 days using Spectrapor 3 dialysis membrane (M_r cut-off of 3500). The phosphorus-containing impurity in unpurified poly(ethylene glycol) is completely removed after the final step, as judged by ^{31}P-NMR.

Sample preparation. Small unilamellar vesicles were prepared by sonication at 15°C, except for DMPC which was sonicated at 30°C. The sonication is followed by centrifugation to remove large vesicles. To produce large unilamellar vesicles in the 0.1–1.0 μm range, the ether injection method of Deamer and Bangham [10] was followed. The lipids in ether solution were injected at a rate of 0.25 ml/min into aqueous buffer at 45°C. The large unilamellar vesicles were pelleted at 6000 $\times$ g and then filtered through a series of 0.4 to 0.1 μm pore polycarbonate membranes, which resulted in a vesicle size distribution centered around 0.12 μm.

Vesicles were mixed with poly(ethylene glycol) solutions to given w/v percentages. All final mixtures contained 10 mM of lipid. The mixtures were incubated at the given temperature for 1 h (except for kinetic experiments) before experimentation.

Electron microscopy. Most samples for freeze-fracture were rapidly frozen in liquid propane by the copper sandwich method [6]. Those samples in high concentrations of poly(ethylene glycol) were frozen in Balzers cups. Procedures for freeze-fracture and negative staining were described previously [6].

Nuclear magnetic resonance (NMR). Procedures for ^{31}P-NMR were described previously [6]. To eliminate any nuclear Overhauser effects, an inverted gated decoupling routine was used. Sweep widths used were 2000 Hz for proton, and 1000 Hz for ^{31}P. Spin-lattice relaxation measurements were obtained by using an inversion-recovery pulsing routine.

Dynamic light scattering. The experiments were performed by measuring the fluctuations in the light scattered at 60° [11]. The light-scattering apparatus was calibrated by measuring the correlation times for monodisperse polystyrene spheres of 30–2000 nm radii (Dow Diagnostics) at known temperatures and medium viscosities to allow the hydrodynamic size of the vesicles to be computed directly from the correlation function. For polydisperse vesicle samples, the average hydrodynamic size was obtained by a second cumulant analysis of the correlation function [12].

10 mM of sonicated lipid vesicles were incubated with poly(ethylene glycol) or other reactants of known concentrations at the given temperatures for 1 h. The reaction was quenched by diluting these mixtures with buffer equilibrated at

the appropriate temperatures to a final reactant concentration of 0.1%, to assure uniform solution viscosities. It must be noted that due to the polydispersity of the fusion products, the average vesicle size is weighted towards the larger ones [12].

Poly(ethylene glycol) binding assays. Small unilamellar vesicles were incubated with varying concentrations of unlabeled poly(ethylene glycol) and a tracer amount of tritiated poly(ethylene glycol) (0.03 μCi) for 30 min at room temperature. The aggregated lipid-poly(ethylene glycol) complex was separated by centrifugation at $100\,000 \times g$ for 30 min. The percent distribution of poly(ethylene glycol) bound to the precipitated lipid vesicles was determined by measurement of radioactivity using a Packard scintillation counter. The total poly(ethylene glycol) bound to the lipid was calculated by multiplying the amount of phospholipids added by the poly(ethylene glycol)/phospholipid ratio in the precipitated lipid-poly(ethylene glycol) complex, to account for the lipid-poly(ethylene glycol) complex retained in the supernatant after centrifugation.

Results

Interaction of poly(ethylene glycol) and other dehydrating agents with egg PC small unilamellar vesicles

The fusion threshold values of egg PC small unilamellar vesicles upon the addition of glycerol, Dextran, sucrose and poly(ethylene glycol) of M_r values between 200 and 6000 were assayed by dynamic light scattering. A time-dependent experiment was performed first to determine the kinetics of the poly(ethylene glycol)-induced reaction. The kinetics of the fusion reaction were followed by observing diluted solutions of egg PC small unilamellar vesicles from 45 or 25% PEG 6000 after various incubation periods from 30 s to 15 h. These percentages of poly(ethylene glycol) were within the fusion range observed from the turbidity measurements [6]. The reaction is shown to be rapid, the final size of the fusion product is reached within 3 min. The rapidity of the fusion is consistent with the 1-min incubation required in cell fusion [3]. From these results, we chose a 1-h incubation time for subsequent experiments. Diluted samples were periodically rechecked at various time-points to assure that equilibration had occurred.

The threshold concentration of PEG 6000 needed for fusion was determined by light scattering as shown in Fig. 1. Aggregation was visually apparent for all initial PEG 6000 concentrations observed (3% and above). Upon dilution, the samples that had been in 35 and 45% poly(ethylene glycol) remained flocculent in appearance, and formed a precipitate. Consistent with the turbidity measurements [6], irreversible increase in vesicle size occurred above 20% poly(ethylene glycol). No noticeable deviation in this fusion curve was observed when purified and dialysed poly(ethylene glycol) were used, contrary to results obtained for cell fusion by Honda et al. [9], who found that an impurity in commercially available poly(ethylene glycol) was necessary to induce fusion. As shown by electron microscopy observation, this increase in vesicle size is a result of fusion and not aggregation (Fig. 2a–f). Large vesicles became appreciable only after treatment of more than 25% PEG 6000 (Fig. 2d), where multilamellar structures were first observed. From the size polydispersity observed,

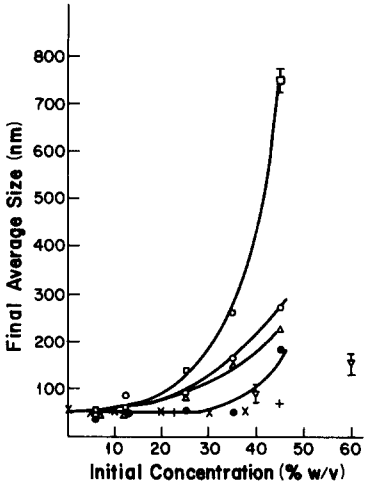
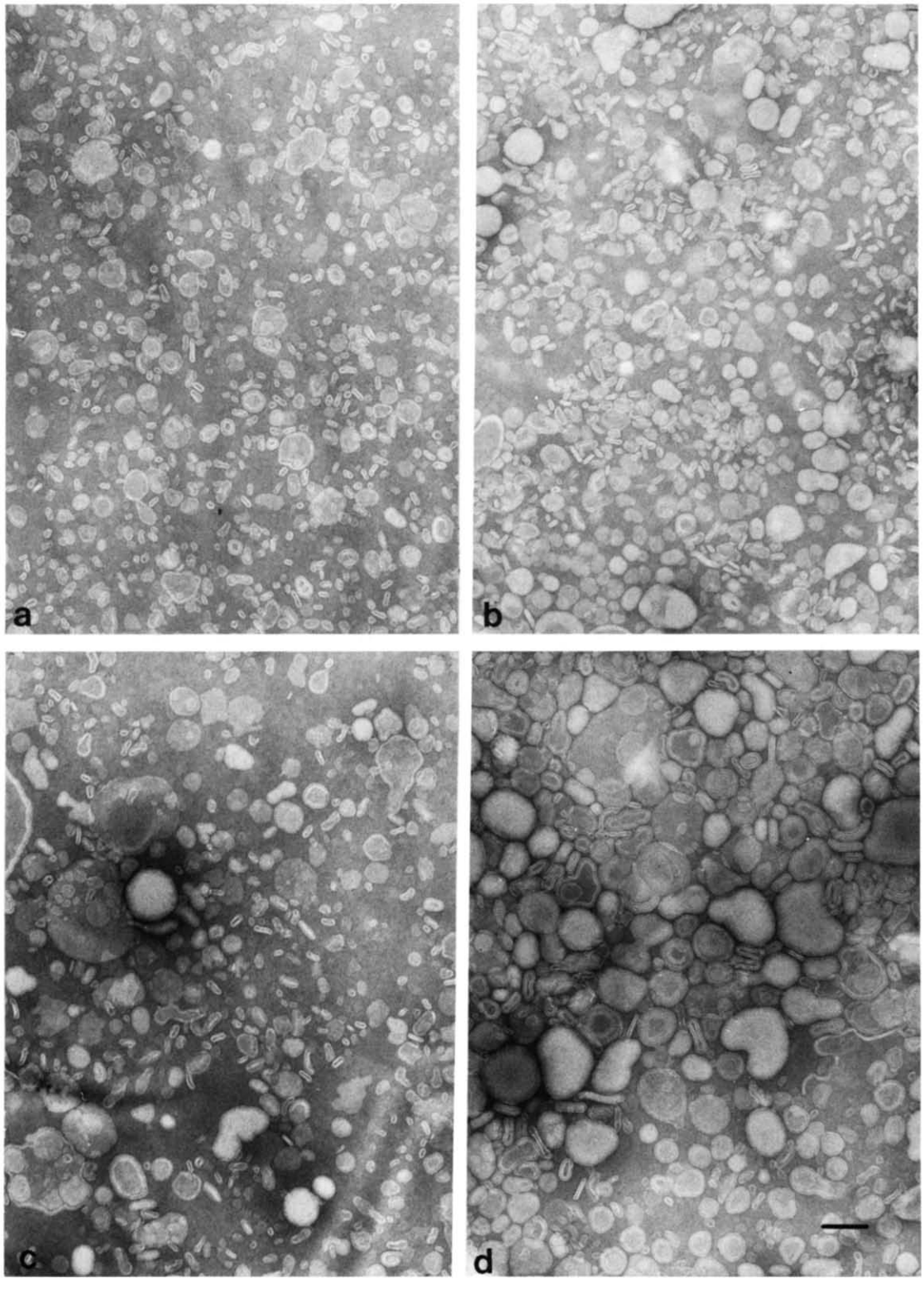


Fig. 1. Fusion induced by poly(ethylene glycol) and analogs, as shown by dynamic light scattering following dilution of 10 mM egg PC small unilamellar vesicles from the given percentage (w/v) of PEG 6000 (□), PEG 1000 (○), PEG 600 (△), PEG 200 (●), ethylene glycol (+), Dextran 200000–300000 (×) and glycerol (▽). No fusion was observed up to 60% sucrose. All data were recorded at 20°C.


a
b
c
d

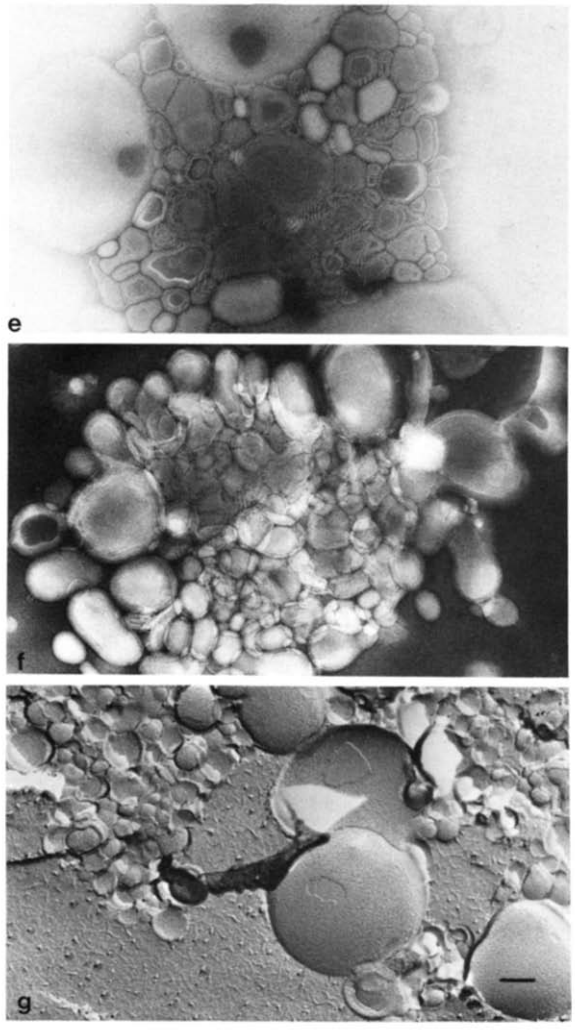


Fig. 2. Fusion induced by PEG 6000 as determined by negative stain and freeze-fracture electron microscopy. The negative stain micrographs show the final vesicle size following dilution from: (a) 0, (b) 6, (c) 12, (d) 25, (e) 35 and (f) 45%. The freeze-fracture micrograph (g) was taken of 10 mM egg PC small unilamellar vesicles in 45% poly(ethylene glycol) 6000, and the sample was frozen in gold Balzer cups. Bar = 0.1 μm.

the results of the light-scattering experiments must be regarded as being a weighted average over a range of vesicle sizes. That fusion takes place prior to dilution in this assaying method is shown in Fig. 2g, a freeze-fracture micrograph of egg PC small unilamellar vesicles in 45% poly(ethylene glycol). Large vesicles with fractures through layers are discernible.

The fusion efficiency of lower molecular weight poly(ethylene glycol), along with other dehydrating agents such as Dextran, glycerol and sucrose [13] were measured likewise. Egg PC small unilamellar vesicles in the various dehydrating agents displayed noticeable turbidity only above 6% PEG 1000, 12% PEG 600, 35% PEG 200, and 5% Dextran, with the rest of the mixtures remaining clear throughout the incubation and dilution. An obvious trend is apparent that a higher fusion efficiency is associated with increasing molecular weight of poly(ethylene glycol) used, in analog to results found for cell fusion by Davidson et al. [14]. No fusion was observed for Dextran, ethylene glycol (Fig. 1) or 60% sucrose (not shown), although some fusion for 60% glycerol did occur (Fig. 1).

The linewidth measurement of proton NMR also supports the electron microscopy and light-scattering results. At concentrations less than 20%, poly(ethylene glycol)-induced line-broadening is reversible upon dilution, indicating the aggregations redisperse, with no increase in vesicle size (results not shown). However, spin-lattice relaxation times for small unilamellar vesicles compared to small unilamellar vesicles in 6% poly(ethylene glycol) showed a decrease from 0.32 to 0.20 s for the N-methyl, 0.39 to 0.25 s for the methylene and 0.58 to 0.33 s for the terminal methyl protons.

The interaction between the phospholipid headgroups and poly(ethylene glycol) or other dehydrating agents was investigated by ^{31}P-NMR. The results are shown in Fig. 3. Upon dilution from 12% poly(ethylene glycol), the line-broadening is reversible (Fig. 3), in agreement with proton NMR results. No anisotropic resonance component was observed in a broadband spectrum of the 18% poly(ethylene glycol)-egg PC, consistent with the vesicles being under 3000 Å in diameter [15]. No significant change, however, was noted in the spin-lattice relaxation rate for small unilamellar vesicles in 6% poly(ethylene glycol) as compared to small unilamellar vesicles in aqueous buffer, both being 1.45 ± 0.05 s. The effects of glycerol and Dextran were compared to that of 6% poly(ethylene glycol), where minimal fusion was observed, thus eliminating an increase in size as a cause of the line-broadening. Only a slight degree of broadening was noted by ^{31}P-NMR for samples in 5 and

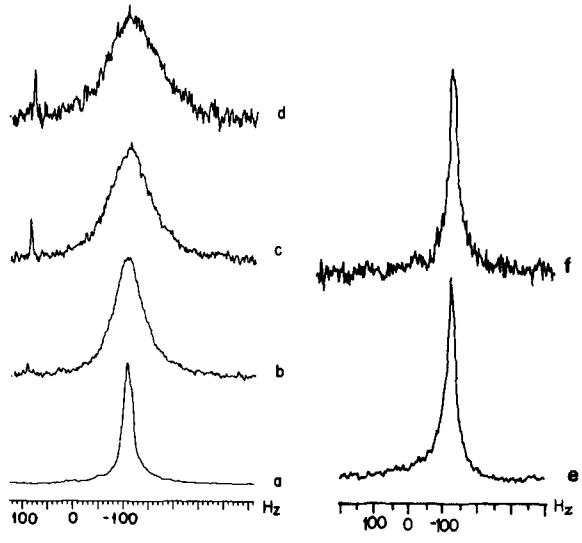


Fig. 3. 81 MHz ^{31}P-NMR spectra of 10 mM egg PC small unilamellar vesicles at 20°C in (a) 0, (b) 6, (c) 12 and (d) 18% PEG 6000. 2000 datum points were collected for over 1500 scans. The reversibility of line-broadening by poly(ethylene glycol) is demonstrated in samples suspended in (f) 3% PEG 6000 after a 4-fold dilution from a suspension in 12% poly(ethylene glycol), as compared to (e) an initial suspension in 3% PEG 6000. Apart from the small peak on the left of (c) and (d), caused by a phosphorus-containing impurity in unpurified poly(ethylene glycol), purified and commercial grade poly(ethylene glycol) give identical spectra.

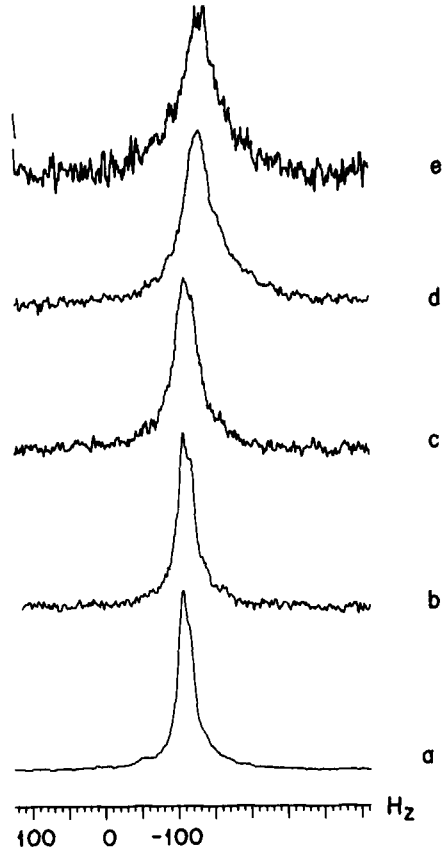


Fig. 4. 81 MHz ^{31}P-NMR spectra of 10 mM egg PC small unilamellar vesicles at 20°C in (a) buffer, (b) 5% Dextran 250000, (c) 10% Dextran 250000, (d) 40% glycerol, and (e) 60% glycerol. Experimental conditions are the same as in Fig. 3.

10% Dextran solutions (viscosity 3.6 and 9.5 cS, respectively) (Fig. 4), when compared to 6% poly(ethylene glycol) (viscosity 3.1 cS). The fact that the Dextran solutions were of higher viscosity, and also turbid in appearance, indicates that poly(ethylene glycol)-induced broadening is more than just a viscosity or aggregation effect. This result is consistent with the lack of broadening in NMR spectra caused by lectin-induced vesicle agglutination [16] or by a highly viscous 1.5% calf thymus DNA solution [17]. The broadening due to 40 and 60% glycerol at respective viscosities 3.3 and 9.3 cS is also less than that caused by poly(ethylene glycol) (Fig. 4).

The NMR results appear to indicate that viscosity and/or aggregation is not enough to cause the broadening observed in poly(ethylene glycol) solutions, unless the poly(ethylene glycol)-induced aggregates were larger than Dextran aggregates, causing a decrease in the tumbling rate. This is shown not to be the case by light-scattering results. On the other hand, decreasing the lateral and rotational diffusion of the lipid molecules would also cause broadening along with a decrease in intensity due to increase in dipolar interactions. This could occur if the poly(ethylene glycol) caused a more gel-like state of the bilayer and/or if the poly(ethylene glycol) was bound to the phospholipids. The poly(ethylene glycol)-induced decrease in spin-lattice relaxation times noted by methylene proton NMR but not ^{31}P-NMR indicates a decrease in the frequency of *trans-gauge* isomerization of the acyl chains, while the phosphorus T-1 is less sensitive to overall motions of phospholipids [18,19].

The ^{31}P-NMR line-broadening induced by poly(ethylene glycol) could be a result of poly(ethylene glycol) interacting with the phospholipid

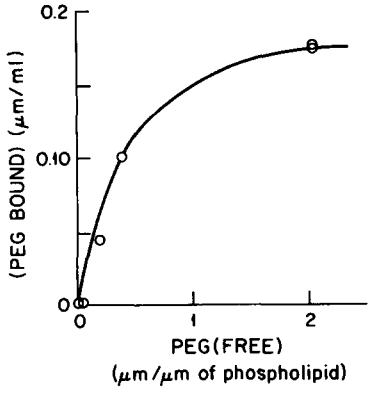


Fig. 5. The binding of PEG 6000 to egg PC small unilamellar vesicles. The curve gives best fit values of $K_d = 6 \cdot 10^{-6}$ M and $B_T = 0.088$ μmol/μmol of phospholipid.

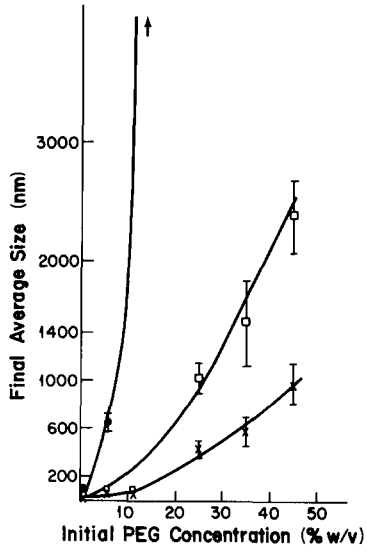


Fig. 6. Dynamic light scattering showing the temperature dependence on fusion for 10 mM DMPC small unilamellar vesicles at 10 (●), 20 (□) and 38°C (×) following dilution from the given initial percent PEG 6000.

headgroup. The binding of poly(ethylene glycol) 6000 to egg PC small unilamellar vesicles is shown in a binding experiment. The binding of poly(ethylene glycol) to the lipid increases with the poly(ethylene glycol) concentration up to 5% of poly(ethylene glycol), when all small unilamellar vesicles are bound and precipitated after centrifugation. The radioactivities due to entrapped supernatant in intervesicular space of pellets, which was less than 5% of the total pellet volume, according to L-glucose space measurements, were subtracted to calculate poly(ethylene glycol) bound. The binding data were analyzed according to Scatchard. The best line fitting the points in Fig. 5 shows an apparent dissociation constant (K_d) of 6 μM and the total binding capacity of 0.088 μmol/μmol of phospholipid, or about 12 molecules of egg PC bind to 1 molecule of poly(ethylene glycol).

The effect of lipid phase transition on the fusion of DMPC small unilamellar vesicles

As measured by dynamic light scattering, DMPC vesicles fuse more readily at 10 than at 20 or 38°C ($T_c = 23.5$°C). A significant increase in vesicle size is seen below the transition temperature (Fig. 6). A slight decrease in fusion efficiency was observed when similar experiments using egg PC were performed at 10°C instead of 20°C (not shown), both of these temperatures are still above the phase transition temperature of egg PC. Since the increase of vesicle size was observed for DMPC and not for egg PC at 10°C, it appears that the increase is more than just a temperature effect. The extent of fusion of DMPC vesicles at 10°C in the presence of poly(ethylene glycol) is much larger than the spontaneous fusion of small unilamellar vesicles in a control sample maintained at 10°C without poly(ethylene glycol) for the same time duration. The degree of fusion of DMPC vesicles above the T_c is comparable to that found for egg PC. The fusion product in all cases were multilamellar vesicles, and revealed the P_β rippled structure as observed in freeze-fracture micrographs (not shown) of pellets from selected samples freeze-quenched at 20°C.

The effect of surface charge on the fusion of bovine PS small unilamellar vesicles

The effect of poly(ethylene glycol) on the charged lipid vesicle fusion is seen by dynamic light scattering in Fig. 7. Bovine PS small unilamellar vesicles in 7 mM Tris-HCl buffer with 3 mM of NaCl are shown to fuse minimally and only at the high poly(ethylene glycol) concentrations. In a buffer solution containing 100 mM NaCl, both aggregation and fusion to a degree comparable to that found for egg PC small uni-

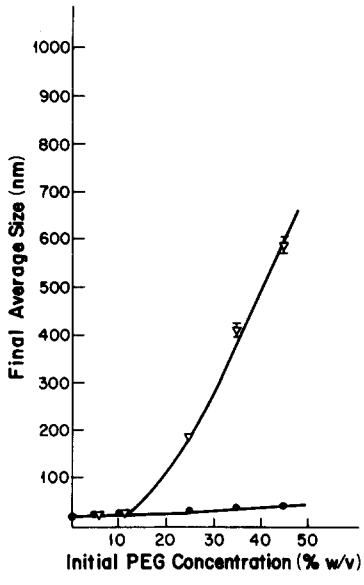


Fig. 7. Dynamic light scattering showing the fusion of 10 mM bovine PS small unilamellar vesicles in salt-free buffer (●) and in buffer containing 100 mM sodium chloride (▽) following dilution from the given initial percent PEG 6000.

lamellar vesicles are observed. The onset of fusion however appeared at a slightly lower poly(ethylene glycol) value and was much sharper. This strong dependence on ionic strength is most likely due to an inability of poly(ethylene glycol) to overcome the repulsion created by the negatively charged PS vesicles unless the vesicles are neutralized by the sodium ions.

Aggregation and fusion of large unilamellar vesicles of egg PC

The high degree of curvature in small unilamellar vesicles may have a significant effect on the molecular packing, which would render small unilamellar vesicles less stable thermodynamically than larger vesicles, and leave them more susceptible to spontaneous fusion to dissipate this excess free energy [20]. It is therefore important to investigate interactions of poly(ethylene glycol) with large vesicles. This measurement was done by freeze-fracture electron microscopy.

A representative freeze-fracture electron micrograph of the large unilamellar vesicles prior to fusion is given in Fig. 8a. Few multilamellar structures are observable. The size distribution, assuming a random fracture through different sections of spherical vesicles, was calculated by the method of Weibel and Bolender [21]. An aliquot of the sample during incubation in 50% poly(ethylene glycol) was frozen and inspected by freeze-fracture microscopy (Fig. 8b). Aggregation is shown to be extensive with tight vesicle attachments, making boundaries between vesicles undiscernable. It is also interesting to note that large unilamellar vesicles in as low as 0.5% poly(ethylene glycol) were clumped together, as revealed by light mi-

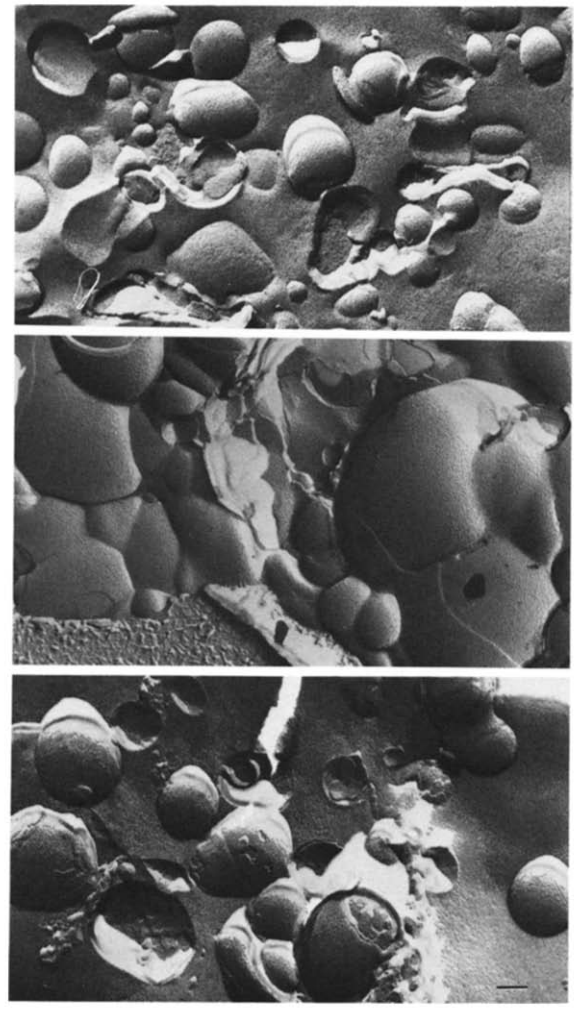


Fig. 8. Freeze-fracture electron micrographs of large unilamellar vesicles of egg PC (a) before poly(ethylene glycol) treatment, (b) during the incubation in 45% PEG 6000 and (c) following a 1-h incubation in 45% poly(ethylene glycol) 6000 with subsequent washing. Bar = 0.1 μm.

croscopy. Such aggregation was not noted by light scattering of small unilamellar vesicles in 0.5% poly(ethylene glycol). The product after poly(ethylene glycol) removal is shown in Fig. 8c. From corresponding histograms of size distributions of vesicles before and after poly(ethylene glycol) treatment, a slight increase in the average size along with a large decrease in the number of smaller large unilamellar vesicles is found. The mode and median diameters shifted from 120 to 200 nm, and from 140 to 220 nm, respectively. These results indicate that poly(ethylene glycol) does induce fusion between large unilamellar vesicles, but not as dramatic as that found for small unilamellar vesicles. This is consistent with results from experiments with calcium-induced PS fusion, where small unilamellar vesicles are found to fuse more readily than large unilamellar vesicles [22].

Discussion

We have shown that poly(ethylene glycol) can cause reversible aggregation of egg PC small unilamellar vesicles at concentrations between 2 and 12%. The aggregation at this low poly(ethylene glycol) concentration is consistent with 0.5–5% poly(ethylene glycol) decreasing the surface potential of DPPC monolayers [23]. By diminishing the electrostatic field perpendicular to the surface of the lipid molecules, extensive aggregation would be expected. An increased efficiency of flocculation of latex spheres with increasing molecular weight poly(ethylene glycol) has also been observed [24] and has been attributed to an adsorption of the neutral polymer onto the dispersed, charged particles. However, an excluded volume effect [25] of poly(ethylene glycol) cannot be ruled out.

Extensive fusion of small unilamellar vesicles occurs above 25% for PEG 6000. This is within the range where all the water is structured [26]. Lower molecular weight poly(ethylene glycols) are less efficient, perhaps due to their lower ability to structure water. Removal of poly(ethylene glycol) is not necessary for the fusion of small unilamellar vesicles. Thus, the mechanism of pure lipid small unilamellar vesicle fusion induced by poly(ethylene glycol) does not require an osmotic swelling, as proposed by Kao et al. [27] and Knutton [3]. Dehydration alone is not sufficient to induce membrane fusion, since the dehydrating agent, Dextran, does not fuse small unilamellar vesicles, as indicated in this study by light-scattering experiments, and has been shown to be incapable to fuse cells [28].

^{32}P-NMR results of small unilamellar vesicles in poly(ethylene glycol) above the T_c reveal a restriction in the motion of the phospholipid molecules that is greater than what would be expected due to viscosity, aggregation or dehydration. The primary cause of NMR line-narrowing for small unilamellar vesicles has been attributed to rapid vesicle tumbling and lateral diffusion of the phospholipids in the plane of the bilayer [19]. This has been disputed by those who claim that the narrowing is due to rapid rotational motion of the phospholipid molecules and *trans-gauche* interconversions along the methylene region, which are in turn due to the structural disorder being greater in small unilamellar vesicles than in large vesicles [16–18]. It is however agreed that line-narrowing is due to more complete averaging of the dipole-dipole interactions. The line-broadening caused by poly(ethylene glycol) seems to be a unique property of poly(ethylene glycol). It holds for all lipids we studied, and not a general effect of viscosity and dehydration. It may be a result of poly(ethylene glycol). It holds for all lipids we studied, and not a general effect of viscosity and dehydration. It may be a result of poly(ethylene glycol) binding to phospholipids.

The rigidization of the phospholipid molecules appears to facilitate fusion, possibly through the creation of defects along the boundaries of domains of different molecular packing. This hypothesis also helps to explain the fact that vesicles are fused by poly(ethylene glycol) more readily at temperatures below the phase transition. Blaurock and Gamble [29] predicted small vesicles to be faceted below the T_c, where the edges could be defect sites. Larrabee [30] also predicted the increased fusion rate below the T_c to be due to vesicles being converted to irregular, amorphous fragments. By aggregating these unstable vesicles through poly(ethylene glycol) treatments, thus increasing the local vesicle concentration, the rate and degree of fusion would be expected to increase

significantly. Our observation that small unilamellar vesicles fused more readily than large unilamellar vesicles also supports this argument.

The mechanism of poly(ethylene glycol)-induced fusion may also involve the hydrated polymer solvating the polar headgroups [31,32] and, by a shearing type force, may create defects and allow for the exposure of hydrocarbon tails. Contact between the tails is then made possible in the highly apolar environment poly(ethylene glycol) creates. The exposure of the hydrocarbon tails to the aqueous medium could be a necessary instability for fusion [30] and is consistent with apolar contacts preceding fusion [33]. The lower surface charge found for small unilamellar vesicles in aqueous two-phase systems of water, Dextran and poly(ethylene glycol) has been explained to be due to a greater exposure of the hydrophobic tails [34], and would also explain why small unilamellar vesicles fuse more readily than larger vesicles.

Thus, the poly(ethylene glycol)-induced fusion may be based on a combination of charge neutralization, dehydration and bilayer defect creation. It is this multifunctional property of poly(ethylene glycol) that distinguishes it from other reagents and lends to its high fusion efficiency.

Acknowledgements

We wish to thank T. Isac for his assistance in preparing vesicle samples, R. Edwards for measuring vesicle sizes from electron micrographs, and Drs. P.L. Yeagle and J. Alderfer for discussion and advice in NMR experiments. This work was supported by grants GM 30969, GM 28120 and GM 24590 from NIH.

References

1 Lucy, J.A. (1978) in Cell Surface Reviews Vol. 5 (Poste, G. and Nicolson, G.L., eds.), pp. 267–304, North-Holland, Amsterdam
2 Maul, G.G., Steplewski, Z., Weibel, J. and Koprowski, H. (1976) In Vitro 12, 787–796
3 Knutton, S. (1979) J. Cell Sci. 36, 61–72
4 Robinson, J.M., Roos, D.S., Davidson, R.L. and Karnovsky, M.J. (1979) J. Cell Sci. 40, 63–75
5 Krahling, H. (1981) Z. Naturforsch. 36, 593–596
6 Boni, L.T., Stewart, T.P., Alderfer, J.L. and Hui, S.W. (1981) J. Membrane Biol. 62, 65–70
7 Tilcock, C.P.S. and Fisher, D. (1982) Biochim. Biophys. Acta 688, 645–652
8 Papahadjopoulos, D. and Miller, N. (1967) Biochim. Biophys. Acta 135, 624–638
9 Honda, K., Maeda, Y., Sasakawa, S., Ohno, H. and Tsuchida, E. (1981) Biochem. Biophys. Res. Commun. 101, 165–171
10 Deamer, D. and Bangham, A.D. (1976) Biochim. Biophys. Acta 443, 629–634
11 Sun, S.-T., Day, E.P. and Ho, J.T. (1978) Proc. Natl. Acad. Sci. USA 75, 4325–4328
12 Bargeron, C.B. (1974) J. Chem. Phys. 61, 2134–2138
13 LeNeveu, D.M., Rand, R.P., Parsegian, V.A. and Gingell, D. (1977) Biophys. J. 18, 209–230
14 Davidson, R.L., O'Malley, K.A. and Wheeler, T.B. (1976) Somatic Cell Genet. 2, 271–280
15 Burnell, E., Van Alphen, L., Verkleij, A. and De Kruijff, B. (1980) Biochim. Biophys. Acta 597, 492–501
16 Curatolo, W., Shipley, G.G., Small, D.M., Sears, B. and Neuringer, L.J. (1977) J. Am. Chem. Soc. 99, 6771–6772
17 Sheetz, M.P. and Chan, S.I. (1972) Biochemistry 11, 4573–4581
18 Horwitz, A.F., Michaelson, D. and Klein, M.P. (1973) Biochim. Biophys. Acta 298, 1–7
19 Finer, E.G. (1974) J. Magn. Resonance 13, 76–86
20 Lawaczeck, R., Kainosho, M. and Chan, S.I. (1976) Biochim. Biophys. Acta 443, 313–330
21 Weibel, E.R. and Bolender, R.P. (1973) in Principles and Techniques of Electron Microscopy. Biological Applications (Hayat, M.A., ed.), Vol. 3, pp. 237–296, Van Nostrand Reinhold Co., New York
22 Nir, S., Wilscut, J. and Bentz, J. (1982) Biochim. Biophys. Acta 688, 275–278
23 Maggio, B. and Lucy, J.A. (1978) FEBS Lett. 94, 301–304
24 Vincent, B. (1974) Adv. Colloid Interface Sci. 4, 193–277
25 Atha, D.H. and Ingham, K.C. (1981) J. Biol. Chem. 256, 12108–12117
26 Baran, A.A., Solomentseva, I.M., Mank, V.V. and Kurilenko, O.D. (1972) Dokl. Akad. Nauk. S.S.S.R. 207, 363–366
27 Kao, K.N. and Michayluk, M.R. (1974) Planta 115, 355–367
28 Ahkong, Q.F., Howel, J.I., Lucy, J.A., Safwat, F., Davey, M.R. and Cocking, E.C. (1975) Nature 255, 66–67
29 Blaurock, A.E. and Gamble, R.C. (1979) J. Membrane Biol. 50, 187–204
30 Larrabee, A.L. (1979) Biochemistry 18, 3321–3326
31 Tilcock, C.P.S. and Fisher, D. (1979) Biochim. Biophys. Acta 577, 53–61
32 Arnold, K., Pratsch, L. and Gawrisch, K. (1983) Biochim. Biophys. Acta 728, 121–128
33 Hui, S.W., Stewart, T.P., Boni, L.T. and Yeagle, P.L. (1981) Science 212, 921–923
34 Eriksson, E. and Albertsson, P.-A. (1978) Biochim. Biophys. Acta 507, 425–432