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INTERACTION OF PHOSPHOLIPID MEMBRANES WITH POLY(ETHYLENE GLYCOL)S

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Summary

1. The water-soluble polymer, poly(ethylene glycol), causes concentration-dependent increases in the temperature of the gel—liquid crystalline phase transitions of aqueous dispersions of dipalmitoyl phosphatidylcholine and of dipalmitoyl phosphatidylethanolamine.

2. For dipalmitoyl phosphatidylcholine it has been further demonstrated that poly(ethylene glycol) increases the transition enthalpy and entropy while decreasing the cooperativity of the transition.

3. These results are discussed in relation to the possible modes of action of poly(ethylene glycol) in promoting cell fusion.

Introduction

Poly(ethylene glycol), in concentrated aqueous solution is very effective in causing biological membranes to fuse [1—10]. We have recently found [11] that the fusion of LN-skin fibroblasts with solutions of poly(ethylene glycol) of various molecular weights is critically dependent upon the concentration of the polymers; fusion being extensive when cells were treated with solutions of 45—50% (w/w), whereas higher concentrations caused cell damage. In addition, poly(ethylene glycol) increased the permeability of chicken erythrocytes to Ca^{2+}, with a dependence upon polymer concentration similar to that observed for cell fusion [11].

Poly(ethylene glycol)s are miscible with water in all proportions [12] and bind approximately their own weight of water [13—16]. We have found that the solutions of poly(ethylene glycol) that are most effective in causing cell

Abbreviations: DPPC, dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl phosphatidylethanolamine.

fusion contain little or no unbound water detectable by differential scanning calorimetry and have considered that the relatively low free water content of such solutions is a property that is important in relation to their ability to cause membrane fusion.

In an attempt to understand how poly(ethylene glycol)s interact with biological membranes, their effects on phospholipids in monolayers have been studied 17,18]. Poly(ethylene glycol)s, together with other water-soluble fusogens such as sorbitol and dimethylsulphoxide, decreased the surface potential of monolayers of DPPC [17] and altered the phase transition temperature [18].

In the present studies the thermotropic properties of aqueous dispersions of DPPC and DPPE were investigated in the presence of various poly(ethylene glycol)s, primarily poly(ethylene glycol) 400. Our observations indicate that solutions of poly(ethylene glycol) 400, particularly those containing greater than 30—35% (w/w) of polymer, cause an increase in the transition temperature of the phospholipids, the effect increasing with increasing concentration of polymer.

Materials and Methods

Poly(ethylene glycol)s. Poly(ethylene glycol) 400, 1500 and 6000 were supplied by BDH (Poole, Dorset).

Lipids. DPPC and DPPE were supplied by Sigma (London) Chemical Company and were shown to be homogeneous by thin-layer chromatography.

Preparation of lipid dispersions. Vesicles of DPPC were prepared by a modification of the method of Batzri and Korn [19]. 75 μl of an ethanolic solution of DPPC (30 mg/ml) were rapidly injected with a Hamiltion syringe (Micromeasure N.V., The Hague, Netherlands) into 1 ml of water at 50°C, the procedure repeated until sufficient vesicles for the experiment were obtained, and the pooled suspensions centrifuged (190 000 $\times g$ for 120 min). The pellet was resuspended in water to give a solution containing approximately 10% (w/w) of lipid; the actual phospholipid concentration was determined by phosphorous analysis [20]. The vesicles were stored at room temperature under nitrogen. The purity of the preparation was checked at intervals by thin-layer chromatography. After 7 days traces (less than 3% of total lipid) of lysophosphatidylcholine and palmitic acid were found, but these contaminants had no detectable effect upon the thermal properties of DPPC, consistent with the findings of Klopfenstein et al. [21]. The mean value (± S.D.) of 16 determinations of the transition temperature of several preparations of vesicles used was 40.9 ± 0.7°C. Although this method has been used for the preparation of single bilayer vesicles [19] indistinguishable from those obtained by sonication, with an average diameter of about 26.5 nm, the vesicles that we obtained appeared larger (approx. 50 nm diameter) than this in freeze-fracture preparations. Since, in addition, the thermal properties resembled those of multilamellar rather than sonicated vesicles [22] we consider that our preparations do not contain a significant number of small unilamellar vesicles.

Multilamellar liposomes were prepared by the method of Bangham et al. [23], the lipid being dispersed as a 20% (w/w) mixture in double-distilled deionised water by heating to 50°C and vortexing for 5 min. The liposomes

were stored at room temperature under nitrogen and their phospholipid concentration determined as described for Batzri and Korn-type vesicles. The mean value (± S.D.) of 15 determinations of the transition temperature of the several preparations used was 41.1 ± 0.5°C.

DPPE was prepared as a 20% (w/w) dispersion in either water or 0.145 M NaCl by repeated vortexing at 70°C. The mean value (± S.D.) of eight determinations of the transition temperature of the several preparations used was 62.6 ± 0.6°C.

Differential scanning calorimetry. Differential scanning calorimetry was performed using a Perkin-Elmer DSC-1B calorimeter in the low temperature mode with liquid nitrogen as coolant. To be able to study samples greater than 20 μl, the maximum volume that can be used in the usual volatile sample holders provided by the manufacturer, specially constructed sample containers of 100 μl capacity were made from brass or duralumin. Known weights of lipid preparations and neat poly(ethylene glycol) 400 or concentrated solution of poly(ethylene glycol) 1500 and 6000 were placed in the pans which were sealed with the usual aluminium lids used for the 20-μl pans. Samples were immediately heated at 4°C/min from 7°C to 10°C above the transition of the phospholipid and then cooled to 7°C. This process was repeated for two further cycles of heating and cooling. In some instances, in order to measure the amount of unbound water in the preparation, the samples were subsequently cooled to −100°C heated to 10°C above the lipid transition and recooled to −100°C. This cycle was also repeated twice. The transition temperature of the lipid was measured to the neareast 0.5°C and was taken as the point of first departure from the baseline for DPPC, but for DPPE, because this lipid exhibited a gradual transition onset which proved difficult to measure accurately in this way, the position of the peak maximum was used. Transition areas were measured by planimetry. The enthalpy of the transition was calculated by the use of a standard curve relating peak area to the transition enthalpy of indium standards and the known weight of lipid in the sample. The entropy of the transition was derived as described by Phillips et al. [24]. The free water content (unbound water), as a percentage of sample weight, was obtained by measuring the peak area due to the melting of ice and relating it to a standard curve of peak area versus weight of water. The sizes of the apparent cooperativity units [25] were determined as the ratio of the van't Hoff enthalpies to the measured calorimetric enthalpies. The van't Hoff enthalpies were derived as described by Mabrey and Sturtevant [26] as $6.9\ (T_m)^2/\Delta T_{1/2}$ where T_m is the mid-point temperature of the transition and $\Delta T_{1/2}$ the width of the transition at half-peak height, although it is recognised that this relationship is strictly applicable only to transitions exhibiting symmetrical excess specific heat profiles [26]. The values of the cooperativity unit (± S.D.) obtained for DPPC in water as multilamellar liposomes was 66.6 ± 9.2 for three samples each measured in triplicate. This value is of the same order as published values of 70 ± 10 [25], 130 [22] and 260 molecules [27]; it should be appreciated that the size of the unit is particularly affected by impurities, instrumental factors and other unrecognised influences [26].

Results

The effect of different concentrations of poly(ethylene glycol) 400 and poly(ethylene glycol) 1500 on the phase transition of DPPC vesicles prepared by the method of Batzri and Korn [19] is shown in Fig. 1. The pretransition was detectable in preparations of the lipid in water but was not pronounced, presumably because diluted lipid samples were used. The pretransition was not detectable in the presence of the lowest concentration of poly(ethylene glycol) 400 used (23.3% w/w). The main transition showed an increase in transition temperature and a broadening of the endotherm with increasing concentrations of polymer. These effects became marked above about 40% poly(ethylene glycol).

In Fig. 2 the increase in transition temperature produced by each concentration of poly(ethylene glycol) is plotted against the polymer concentration. In this diagram the unbound water composition of the solutions is also shown. In solutions containing poly(ethylene glycol) 400 at greater than 52% (w/w) no water was unbound.

Although poly(ethylene glycol) 400 causes the fusion of fibroblasts [11], it is not the most fusogenic of the poly(ethylene glycol)s that we have studied. However, it was chosen for the present studies because it is a liquid at room temperature, which made it easier to handle than the polymers of higher molecular weight studied which are solids (poly(ethylene glycol) 1500, 4000, 6000 and 20 000) or semi-solid (poly(ethylene glycol) 600). To demonstrate that the

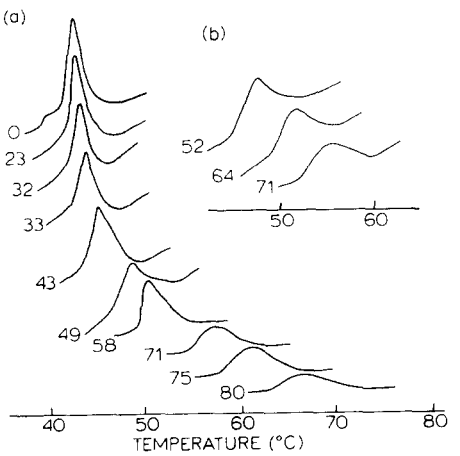

Fig. 1. Differential scanning calorimetry of vesicles of DPPC prepared by the method of Batzri and Korn [19], treated with (a) poly(ethylene glycol) 400 and (b) poly(ethylene glycol) 1500. The concentrations shown are the final polymer concentrations (%, w/w). The samples contained varying amounts of phospholipid so that direct comparisons of the size of transition areas between samples are not appropriate.

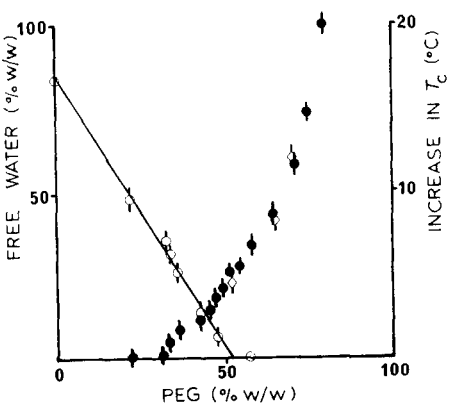

Fig. 2. The effect of poly(ethylene glycol)s on the transition temperature of vesicles of DPPC prepared by the method of Batzri and Korn [19] and on the free water content of the solution. The transition temperatures of the vesicles were measured in water and in the presence of polymer; the increase in transition temperatures caused by poly(ethylene glycol) 400 (•) and poly(ethylene glycol) 1500 (◇) are shown together with the free water content (○) of the mixture of poly(ethylene glycol) 400 and vesicles. Each point is the mean (± S.D.) of a sample measured in triplicate.

results obtained are not just a property of poly(ethylene glycol) 400 we have studied the effect of poly(ethylene glycol) 1500 and have obtained essentially similar results (Fig. 1 and 2).

An increase in transition temperature was also obtained when multilamellar liposomes of DPPC were treated with increasing concentrations of poly(ethylene glycol) 400 (Fig. 3), although in this case the polymer appeared more effective at lower concentrations. A less detailed study was carried out with poly-(ethylene glycol) 6000, but as can be seen from Fig. 3a and b, the results obtained were very similar to those obtained with poly(ethylene glycol) 400. In the studies with multilamellar liposomes the pretransition was more apparent than in the case of Batzri and Korn-type vesicles. It occurred at 35.8 ± 0.6°C (three determinations) for preparations of the liposomes in water. In the presence of 8.6% (w/w) poly(ethylene glycol) 6000 the pretransition endotherm was shifted to 38.5 ± 0.3°C (four determinations) whereas in 18.1% (w/w) poly(ethylene glycol) 6000 it had been eliminated.

The differential scanning calorimetry traces obtained with the multilamellar liposomes were sufficiently symmetrical for measurements of the enthalpy and entropy of the transitions to be made (Fig. 3b). While individual values often showed considerable variation, the results indicate that there is an increase in both the enthalpy and entropy of the transition with increasing concentrations of polymer. Above 30% (w/w) and up to 95% (w/w) the values appeared approximately constant and the mean values (± S.D.) for the 20 different concentrations of polymer studied were $\Delta H = 10.1 \pm 0.6$ kcal · mol^{-1} and $\Delta S = 31.3 \pm 1.8$ cal · deg^{-1} · mol^{-1}. These values were increased significantly ($P > 0.001$) over the values obtained for four samples of the lipid in water in the

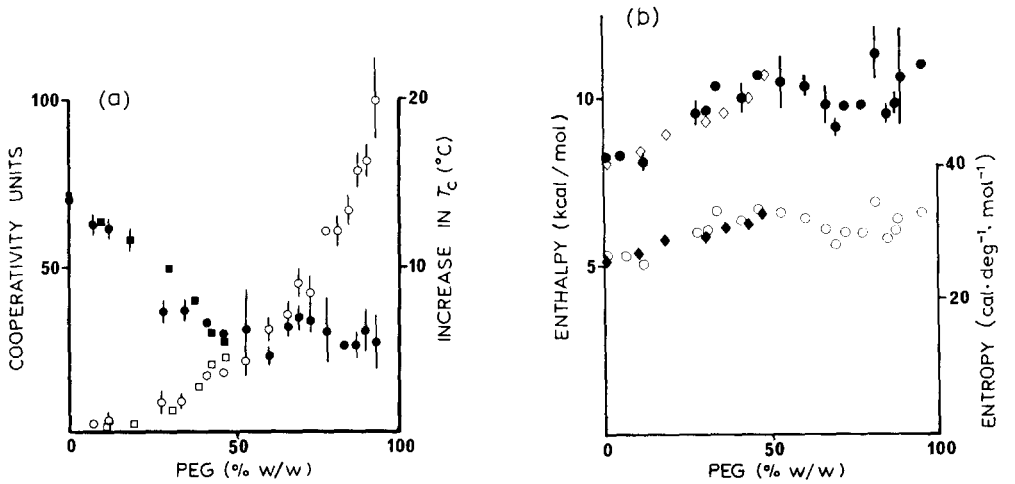

Fig. 3. The effect of poly(ethylene glycol) 400 and poly(ethylene glycol) 6000 on the gel—liquid crystal transition of multilamellar vesicles of DPPE. (a) The increase in transition temperature for poly(ethylene glycol) 400 (○) and poly(ethylene glycol) 6000 (□) is given as the mean (± S.D.) of the difference between the transition temperature observed in the presence of the polymers and in their absence, each measured in triplicate. The sizes of the cooperativity units for poly(etylene glycol) 400 (●) and poly(ethylene glycol) 6000 (■) are shown as the mean (± S.D.) for the same samples. (b) The enthalpy values for poly(ethylene glycol) 400 (●) and poly(ethylene glycol) 6000 (◇) and entropy values for poly(ethylene glycol) 400 (○) and poly(ethylene glycol) 6000 (◆) are the mean values (± S.D.) of the samples.

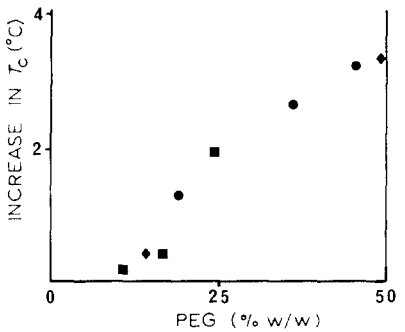

Fig. 4. The effect of poly(ethylene glycol) 400 and 1500 on the transition temperature of dispersions of DPPE. The increases in transition temperature are shown for dispersions prepared in water (■,◆) and in 0.145 M NaCl (●) treated with poly(ethylene glycol) 400 (◆) and poly(ethylene glycol) 1500 (●,■).

absence of polymer (ΔH = 8.3 ± 0.2 kcal · mol^{-1}; ΔS = 26.4 ± 0.6 cal · deg^{-1} · mol^{-1}). Van Dijck [28] has noted that ethylene glycol, the monomer of poly(ethylene glycol), causes increases in the temperature as well as the enthalpy of the phase transition of DPPC.

As observed for vesicles prepared by the method of Batzri and Korn [19], the peaks obtained with multilamellar liposomes were progressively broadened with increasing poly(ethylene glycol) concentration, indicating that the cooperativity of the transition was being decreased. Fig. 3a shows that the size of the cooperativity unit for the transition decreased from about 67 molecules, for the liposomes in water, to about 30 molecules for the liposomes in 50% (w/w) poly(ethylene glycol).

The effects of poly(ethylene glycol)s upon DPPE were also studied since it has been found that lipid-soluble fusogens interact with DPPC under certain conditions whereas they show little specific interaction with phosphatidylethanolamines [29,30]. Dispersion of DPPE in water or 0.145 M NaCl, when treated with poly(ethylene glycol) 400 or poly(ethylene glycol) 1500, showed increases in transition temperature (Fig. 4) similar, over the limited range studied, to those obtained with DPPC.

Discussion

It is clear that poly(ethylene glycol)s can markedly increase the transition temperature of DPPC and alter the cooperativity, enthalpy and entropy of the transition.

At the present time we are unable to give a precise interpretation in molecular terms of these effects of poly(ethylene glycol)s. However, since the transition temperature of DPPC is critically dependent on the polar head group being fully hydrated, a possible explanation for the increase in transition temperature in solutions containing greater than 52% (w/w) poly(ethylene glycol), in which no free water is detected by differential scanning calorimetry, is that the polymer competes with polar head group of the lipid for the bound water in the system, the polar head group is less than fully hydrated and the lipid transition temperature consequently increased. However, if this were the case it

would also be expected that the enthalpy of transition would approach that of the monohydrate (4.55 kcal · mol^{-1}, T_c = 65°C, [24]) and of the anhydrous compound (4.3 kcal · mol^{-1} [31]). In fact the value of ΔH obtained above 30% (w/w) poly(ethylene glycol) was 10.1 kcal · mol^{-1}. In addition it should be noted that in the concentration range of polymer up to 52% (w/w) the transition temperature was increased by about 4°C. The polar head groups of the lipid should still be fully hydrated since unbound water is detectable up to 52% (w/w) poly(ethylene glycol). This small shift in transition temperature cannot therefore be explained simply on the basis of dehydration of the polar head group. However, the polymer may well be able to affect more water molecules than it binds and may alter the chemical activity of water molecules. It has been suggested that poly(ethylene glycol)s may exert a 'structuring action' on several molecular water layers surrounding the polymer, which may involve up to 16 water molecules/monomer unit [32]. Thus all the water would be 'structured' in solutions that were greater than about 13% (w/w).

An alternative explanation for these observations may lie in not considering water as the only component of the polymer solutions that is capable of solvating the polar head group of the phospholipid. It is possible that the hydrated polymer and the partially hydrated polymer may also be able to solvate the polar head group and that the increase in transition temperature, enthalpy and entropy reflect changes in the polymer solvent rather than simply a decrease in the free water in solution. However, since we have found that sonicated of DPPC are aggregated by even dilute solutions of poly(ethylene glycol) 6000 (Tilcock, C.P.S. and Fisher, D., unpublished observation), the alterations in the characteristics of the thermal transition of the lipid described here may reflect the environment into which the vesicles are excluded rather than the effect of the polymer solvent upon the lipid.

There have been several interpretations of the molecular events that give rise to the pretransition of disaturated phosphatidylcholines [33—36]. Janiak et al. [33] consider that the water matrix surrounding the phospholipid head group is necessary for the appearance of the pretransition and suggest that the elimination of the pretransition in 50% (v/v) ethylene glycol observed by Klopfenstein et al. [21] is a consequence of a disruption of this water matrix. Poly(ethylene glycol) may act in a similar manner. Yi and MacDonald [36] have suggested that the pretransition is associated with aggregation-disaggregation reactions between liposomes. Since poly(ethylene glycol) causes phosphatidylcholine liposomes to aggregate, the elimination of the pretransition could be explained, on the basis of this suggestion, as arising from an alteration of aggregation-disaggregation reactions in favour of a non-reversible aggregation.

In the studies described here the poly(ethylene glycol) has been added to preformed liposomes. If the action of the polymer were confined to the outer bilayer of multilamellar liposomes, it might be expected that the differential scanning calorimetry traces would show two peaks, arising from the outer bilayer, the transition temperature of which would be raised, and from the inner bilayers which would remain unaffected. Such a result was never observed. It thus appears that the action of the polymer is not confined to the outer bilayer. There are several possible explanations for this observation. Firstly, the

membrane might be permeable to the polymers, but although this is probable for poly(ethylene glycol) 400, it seems unlikely for the larger polymer poly(ethylene glycol) 6000. Secondly, it is possible that the poly(ethylene glycol) may cause the membranes to break open and expose all the bilayers to polymer.

Since poly(ethylene glycol) alters the solvation properties of water so as to render the solvent less polar [30], it is possible that poly(ethylene glycol) solutions decrease the hydrophobic interaction necessary for the stabilisation of the lipid bilayer. In addition, when applied to the extracellular face of a cell membrane, poly(ethylene glycol) solutions may induce an at least transitory osmotic gradient across the membrane, which may promote pore formation, as has been suggested in the case of lipid vesicles composed of DPPC [38].

Poly(ethylene glycol)s appear similarly to cause increases in the transition temperature of DPPE. Recent studies (Tilcock, C.P.S. and Fisher, D., unpublished results) of the interaction between poly(ethylene glycol) 400 and mixed liposomes composed of DPPC and DPPE in a 1 : 1 molar ratio, indicated that addition of poly(ethylene glycol) to such liposomes results in an elevation of the transition temperature, similar to that observed for the individual lipid components. These results contrast with those obtained by Maggio and Lucy [18] for monolayers of phospholipids treated with poly(ethylene glycol) 1500 as well as poly(ethylene glycol) 6000, $3 \cdot 10^5$ and $5 \cdot 10^6$. They found that whereas monolayers of DPPC behaved as if the transition temperature had been raised, those of DPPE behaved as if it had been depressed.

The interaction of poly(ethylene glycol)s with cell membranes is likely to be more complex than with liposomes. However, the studies presented in this paper suggest that an important feature of the action of poly(ethylene glycol) on cell membranes might be to raise the transition temperature of some, if not most, of the membrane phospholipids. Although an increase in membrane fluidity has been considered to be important for the fusion of cells by lipid-soluble fusogens [39,40] and of liposomes [41], it has been suggested [42] that the fusion of phospholipid vesicles containing negatively charged phospholipids such as phosphatidylserine, phosphatidylglycerol or phosphatidic acid, produced by Ca^{2+}, is a result of Ca^{2+} raising the transition of temperature of the negatively charged lipids and causing a transient increased permeability to Ca^{2+}. The increase in transition temperature produced by poly(ethylene glycol) might be similarly effective in causing lipid bilayers to fuse and might be the reason for the increased permeability of Ca^{2+} observed in erythrocytes treated with poly(ethylene glycol) [43].

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References

1 Kao, K.N., Constabel, F., Michayluck, M.R. and Gamborg, O.L. (1974) Planta 120, 215—227
2 Wallin, A., Glimelius, K. and Erikkson, T. (1974) Z. Pflanzenphysiol. 74, 68—80
3 Ahkong, Q.F., Fisher, D., Tampion, W. and Lucy, J.A. (1975) Nature 253, 194—195
4 Maggio, B., Ahkong, Q.F. and Lucy, J.A. (1976) Biochem. J. 179, 647—650
5 Hartmann, J.X., Galla, J.D., Emma, D.A., Kao, K.N. and Gamborg, O.L. (1976) Can. J. Genet. Cytol. 18, 503—512
6 Ahkong, Q.F., Howell, J.I., Lucy, J.A., Safwat, F., Davey, M.R. and Cocking, E.C. (1975) Nature 255, 66—67
7 Pontecorvo, G. (1975) Somatic Cell Genet. 1, 387—400
8 Pontecorvo, G. (1976) in Baltimore Conference 1975: 3rd Int. Workshop on Human Gene Mapping, Original Article Series, pp. 399—400, National Foundation, New York
9 Davidson, R.L., O'Malley, K.A. and Wheeler, T.H. (1976) Somatic Cell Genet. 2, 271—280
10 Pontecorvo, G., Riddle, P.N. and Hales, A. (1977) Nature 265, 257—258
11 Blow, A., Botham, G., Fisher, D., Goodall, A.H., Tilcock, C.P.S. and Lucy, J.A. (1978) FEBS Lett. 94, 305—310
12 Bailey, F.E. and Koleske, J.V. (1976) Poly(ethylene oxide), Academic Press, New York
13 Molyneux, P. (1975) in Water: A Comprehensive Treatise (Franks, F., ed.), Vol. 4, pp. 569—757, Plenum Press, New York and London
14 Zinchenko, V.D., Mank, V.V., Moiseev, V.A. and Ovchavenko, F.D. (1976) Kolloidn. Z. 38, 44—50
15 Liu, K-J. and Parsons, J.L. (1969) Macromolecules 2, 529—533
16 Maxfield, J. and Shepherd, I.W. (1975) Polymer 16, 505—509
17 Maggio, B., Ahkong, Q.F. and Lucy, J.A. (1976) Biochem. J. 158, 647—650
18 Maggio, B. and Lucy, J.A. (1978) FEBS Lett. 94, 301—304
19 Batzri, S. and Korn, E.D. (1975) J. Cell Biol. 66, 621—634
20 Baginski, E.S., Foa, P.P. and Zak, B. (1967) Clin. Chem. 13, 326—331
21 Klopfenstein, W.E., de Kruyff, B., Verkleij, A.J., Demel, R.A. and van Deenen, L.L.M. (1974) Chem. Phys. Lipids 13, 215—222
22 Sturtevant, J.M. (1974) in Quantum Statistical Mechanics in the Natural Sciences (Kursunoglu, B., Mintz, S. and Widmayer, S., eds.), pp. 63—84, Plenum Press, New York
23 Bangham, A.D., Standish, M.M. and Watkins, J.C. (1965) J. Mol. Biol. 13, 238—252
24 Phillips, M.C., Williams, R.M. and Chapman, D. (1969) Chem. Phys. Lipids 3, 234—244
25 Hinz, H-J. and Sturtevant, J.M. (1972) J. Biol. Chem. 247, 6071—6075
26 Mabrey, S. and Sturtevant, J.M. (1978) in Methods in Membrane Biology (Korn, E.D., ed.), Vol. 9, pp. 237—214, Plenum Press, New York and London
27 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3862—3866
28 Van Dijck, P.W.M. (1977) Thesis, State University Utrecht
29 Maggio, B. and Lucy, J.A. (1975) Biochem. J. 149, 597—608
30 Howell, J.I., Fisher, D., Goodall, A.H., Verrinder, M. and Lucy, J.A. (1973) Biochim. Biophys. Acta 332, 1—10
31 Ladbrooke, B.D. and Chapman, D. (1969) Chem. Phys. Lipids 3, 304—357
32 Baran, A.A., Solomentseva, I.M., Mank, V.V. and Kurilenko, O.D. (1972) Dokl. Akad. Nauk. SSSR 207, 363—366
33 Janiak, M.J., Small, D.M. and Shipley, G.G. (1977) Biochemistry 15, 4575—4580
34 Rand, R.P., Chapman, D. and Larsson, K. (1975) Biophys. J. 15, 1117—1124
35 Larsson, K. (1977) Chem. Phys. Lipids 20, 225—228
36 Yi, P.M. and MacDonald, R.C. (1973) Chem. Phys. Lipids 11, 114—134
37 Ingham, K.C. (1977) Arch. Biochem. Biophys. 184, 59—68
38 Taupin, C., Dvolaitzky, M. and Santerey, C. (1975) Biochemistry 14, 4771—4775
39 Ahkong, Q.F., Cramp, F.C., Fisher, D., Howell, J.I., Tampion, W., Verrinder, M. and Lucy, J.A. (1973) Nat. New Biol. 242, 215—217
40 Fisher, D. (1975) in Biomembranes, Lipids, Proteins and Receptors (Burton, R.M. and Packer, L., eds.), pp. 75—93, BI-Science Publications Division, Webster Groves, MO
41 Papahadjopoulos, D., Posté, G., Schaeffer, B.E. and Vail, W.J. (1974) Biochim. Biophys. Acta 352, 10—28
42 Papahadjopoulos, D., Vail, W.J., Newton, C., Nir, S., Jacobson, K., Poste, G. and Lazo, R. (1977) Biochim. Biophys. Acta 465, 579—598
43 Blow, A.M.J., Botham, G.M. and Lucy, J.A. (1978) Biochem. Soc. Trans. 6, 284—285