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The interaction and fusion of bilayers formed from unsaturated lipids

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Abstract. The interactions between unsaturated phospholipid bilayers deposited on mica were measured in aqueous solution using a surface forces apparatus. The bilayers were made of L-α-dioleoylphosphatidylcholine (DOPC), L-α-dioleoylphosphatidyl ethanolamine (DOPE), and mixtures of the two, and were formed on mica by Langmuir-Blodgett deposition after the lipids were spread on an aqueous substrate from a chloroform solution. The forces are interpreted as electrostatic double-layer and van der Waals forces with long range, and a strong repulsion (hydration or steric force) at distances of several nm. Together they produce a region of weak attraction (a secondary minimum) at 5 nm (DOPE) and 6 nm (DOPC). Fusion of two bilayers into one was observed when the local force per unit area was 2–3 MPa. Other researchers report that phosphatidylethanolamine in vesicles enhances fusion. In this study using deposited bilayers, the presence of DOPE in a DOPC bilayer did not promote fusion, nor did DOPE bilayers fuse more easily than DOPC. The value of the force per unit area at which the two bilayers fuse into one was however decreased by several orders of magnitude when the bilayers were formed from lipids kept in chloroform solution for several days or more. Chromatography showed traces of lipid degradation products in such chloroform solutions.

Key words: Fusion – Unsaturated lipids – Inter-membrane forces – Surface forces apparatus

Introduction

The fusion of membranes is believed to occur in many cellular processes including endocytosis, exocytosis and fertilization. Close approach and interaction between membranes is assumed to be a precursor to fusion. Close approach occurs in biological cells which are dehydrated either by dry atmospheres or by freezing. In these cases, the response of the membranes to the strong intermembrane forces may determine the extent of damage in the cell.

The study of the interaction and fusion of lipid bilayers as a model of biological fusion is motivated by the relative simplicity of such membranes and by the observation that fusion in biological membranes often occurs in regions without membrane particles, i.e. in the putative bilayer matrix of the membrane (Ahkong et al. 1975; Cullis and Hope 1978; Knutton 1979). In order to fuse, two membranes must first come close together, and therefore overcome repulsive forces between them. The repulsion between lipid bilayers is strongly dependent on the lipid composition (Lis et al. 1982). The aim of this study was to examine the inter-membrane repulsion and fusion of membranes.

One model of membrane fusion, first reported by Horn (1984), is that between lipid bilayers deposited on molecularly smooth mica surfaces in aqueous solution. The advantages of this model are that the force and separation between the surfaces can be accurately measured (Israelachvili and Adams 1978) and that the process of fusion can be viewed by optical interferometry as it occurs. The chief disadvantage is the use of mica supports for the bilayers. First, the bilayers are not free to undulate. Second, the fusion proceeds from two parallel bilayers to one, but not usually to zero [1]. As Horn points out, however, such an intermediate stage is suggested by electron micrographs of several secretory structures (Pinto de Silva and Nogueira 1977).

The Israelachvili and Horn technique has been used to study interactions of bilayers of several types of lipids

Abbreviations: DO-, dioleoyl-; PE, phosphatidyl ethanolamine; PC, phosphatidylcholine; FECO, fringes of equal chromatic order

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[1] To make the distinction between the fusion of two bilayers into one and that of two bilayers into zero, the former is sometimes called "hemifusion" or "monolayer fusion", while the latter is called "full fusion" or "bilayer fusion" (Israelachvili and Helm 1989)

(Marra and Israelachvili 1985; Israelachvili and Helm 1989) but to date there has been no study of unsaturated species. Unsaturated lipids are common in plant membranes. In the plasma membrane of rye protoplasts, for example, unsaturated PC and PE are two of the largest classes of lipids present (Lynch and Steponkus 1987). The studies reported in this paper use the technique of Israelachvili and Horn to study the fusion between bilayers of DOPC, of DOPE and of mixtures of the two lipids.

When lipid bilayers are brought close together so that the average water thickness between them is a few nm, then a strong interaction is observed (Lis et al. 1982). It is repulsive for most lipids studied. Two theories compete to explain this force: Marčelja and co-workers attribute it to polarization of the solvent by surface dipoles (Gruen and Marčelja 1979; Kjellander and Marčelja 1985) whereas Israelachvili and Wennerström (1990) and de Gennes and Pincus (1990) attribute it to movements of the lipid molecules. Having friends on both sides, we shall call it the hydration-steric interaction throughout this paper. The force decreases approximately exponentially with separation, and has a characteristic length of typically 0.25 nm. At small separations this force is much larger than other inter-membrane interactions (electrostatic and van der Waals forces). The magnitude of the repulsion at a given distance varies among lipid species over more than an order of magnitude (Lis et al. 1982; Marra and Israelachvili 1985). Thus, if bilayers are to fuse, they must be brought close together against a repulsion which varies strongly with composition.

We chose to investigate DOPC and DOPE bilayers for two reasons. First, we anticipated a substantial difference in the interbilayer repulsion for these lipids. We also considered that the different molecular geometries of these species might be important in fusion. Phosphatidylcholine is a lipid which forms bilayers and which is found in relatively high concentrations in many membranes of plants and animals. Phosphatidylethanolamine is usually found in smaller concentrations but, of the lipids that can form non-lamellar phases, unsaturated PE is one of the most common. Non-bilayer configurations have been implicated in fusion (de Kruijff et al. 1985) and we therefore wished to investigate the effect of such a lipid. The mode of action of fusion-promoting lipids is not known, but two possibilities merit consideration: (*i*) does the fusion depend on a reduction in the short range repulsion between the opposed bilayers? or (*ii*) does the fusion-promoting lipid facilitate the geometric deformations which are intermediate stages in fusion? The surface forces apparatus allows direct measurement of the repulsion, and thus yields information which can help distinguish between these possible explanations.

Most measurements of inter-bilayer forces using the surface forces apparatus have been made on lipids which are solid[2] at room temperature, whereas biological bilayers are fluid under normal conditions. We use dioleoyl lipids because we expected that bilayers of both DOPC and DOPE deposited on mica would be liquid at room temperature. Further they are members of the relatively small class of unsaturated lipids which are available commercially in pure form.

Bilayers with a wide range of surface densities can be deposited on hydrophilic surfaces by varying the lateral pressure of the monolayer at the air-water interface from which they are derived. The appropriate lateral pressure in a monolayer for the deposition of a bilayer which resembles a free membrane is not simply determined[3]. Marra and Israelachvili (1985) have studied the interaction of bilayers deposited onto mica surfaces from monolayers subject to lateral pressures of about 35 $mN \cdot m^{-1}$ (with differences for different lipid species). They argue that this condition produces surface densities which equal those of a bilayer in excess water. It is difficult, however, to determine accurately the pressure at which is deposited a monolayer of appropriate density because the area density of fluid bilayers in excess water is not known accurately. Further there are technical difficulties in depositing homogeneous monolayers at lateral pressures approaching that which collapses the monolayer.

In all cases reported here, the first monolayer deposited on the mica surface was DOPE at 35 $mN \cdot m^{-1}$. This gives a stable, homogeneous hydrophobic surface, and allows comparison with previous results.

The measurements of force were conducted in an aqueous solution of 1 $mol \cdot m^{-3}$ NaCl at pH of approximately 6 (uncontrolled). The strong inter-bilayer repulsion at close approach (hydration-steric force) is at most weakly dependent on a range of dissolved ions (Marra 1985) although electric double layer interactions may depend weakly on the pH. The surface density of monolayers of phosphatidylcholines is at most a very weak function of pH of the subphase at the surface pressure used here (Birdi 1987).

Materials and methods

The forces and separations between surfaces were measured using a technique described by Israelachvili and Adams (1978) in which molecularly smooth mica sheets, partially silvered on one surface, are glued to two cylindrical glass lenses (radius typically 20 mm) whose axes are at right angles. One of the lenses is mounted on a lever spring system and the other on a piezo-electric crystal. The two silvered surfaces form an interferometer to produce fringes of equal chromatic order (FECOs) in white light and give a resolution of relative position of about $\pm 0.1-0.2$ nm. The deflection of the spring as the surfaces approach is calibrated to give the force with a precision of about 2%. The white light was filtered so that the lipid bilayers were exposed only to the green part of the visible spectrum which was the one useful for the measurements.

The water used was obtained from a MilliQ-Organex fed by reverse osmosis water. The lipids used were bought

[2] We use the nomenclature liquid and solid following the suggestion of Pallas and Pethica (1985)

[3] A considerable range of monolayer pressures (12.5 to 50 $mN \cdot m^{-1}$) have been suggested as appropriate for comparision with bilayers (Albrecht et al. 1978; Nagle 1976; Gruen and Wolfe 1982)

from Avanti Polar Lipids and the nominal purity was >99%. Ethanol and chloroform were Merck A. R. grade.

For deposition experiments, the lipids were dissolved in chloroform to give solutions with a concentration of 10^{24} molecules m^{-3}. They were then spread on the air-water interface of a Langmuir trough. All glassware was cleaned in sulphochromic acid and rinsed in Millipore water. Stainless steel and teflon surfaces were cleaned either in sulphochromic acid and water or in alcohol and dried in a jet of nitrogen.

The depositions were conducted and the isotherms were measured in a Teflon coated trough. All surfaces in contact with water were Teflon except for the points of two stainless steel needles used to secure a floating teflon barrier used to measure the surface pressure. The sensitivity in measurement of surface pressure was 0.1 $mN \cdot m^{-1}$ but the calibration was accurate only to 1 $mN \cdot m^{-1}$. For deposition, the barrier was moved by a small servo motor (on anti-vibration mounts) to maintain constant lateral pressure within 0.1 $mN \cdot m^{-1}$. The though was used in a laminar flow cabinet in which the temperature was 22 ± 1 °C and the humidity was not controlled. To determine the surface densities deposited on mica, freshly cleaved sheets of mica with a surface area of about 3000 mm^2 were raised and lowered (for first and second monolayers respectively) through the interface at 10 mm per minute, and the area swept by the barrier was measured. The same speed, 10 mm per minute, was used for deposition of the monolayers. This speed was chosen to maximise homogeneity of the monolayer. Homogeneity was checked by exposing monolayer-coated mica samples to steam, and looking for inhomogeneities in the pattern of the water condensation on the monolayer. At speeds of 10 mm per minute, areas greater than several square millimetres were produced with no observable inhomogeneity.

Lipid bilayers were deposited on the unsilvered side of the exposed mica surfaces by passing the lenses through lipid monolayers at the air-water interface first from water to air, then from air to water. To minimize the contamination of the monolayers on the trough by other surfactants, the surface was rinsed in the following way. First, the trough was cleaned and filled and the interface aspired. Then a monolayer of the desired composition was spread. This monolayer was then compressed and aspired. A further monolayer of the same composition was spread, compressed and aspired. The final monolayer for deposition was then spread and the chloroform was allowed to evaporate for 15 min.

The forces apparatus was filled with 1 $mol \cdot m^{-3}$ NaCl solution which had been degassed by both heating and pumping. The lenses bearing the bilayers were transferred to the apparatus while constantly immersed in aqueous solution in small beakers. An amount of concentrated chloroform solution of lipids (in the same ratio as the outer bilayer) was deposited on the surface of the solution in the apparatus to introduce approximately 10^{16} lipid molecules – more than sufficient to provide a collapsed monolayer on the air-water interface in the apparatus and to saturate the aqueous solution.

Results and discussion

Deposition of monolayers

The first layer deposited on the mica was in all cases drawn from a monolayer of DOPE at an air-water interface with a surface pressure of 35 $mN \cdot m^{-1}$. To maintain this surface pressure in the trough, the reduction in the area of the monolayer was 1.05 ± 0.03 times the surface area of the mica passed through it. Thus we assume that the surface density of the monolayer on the mica was 1.05 times that of the monolayer at the air-water interface. This contraction is small compared to that which accompanies the liquid-solid transition in monolayers at the air-water interface (Albrecht et al. 1978) so it seems to us unlikely that this small reduction in area per molecule implies a condensation from the liquid to the solid phase. When the second monolayer was deposited, the area per molecule in this outer layer was greater than that in the monolayer at the air-water interface. When the monolayer pressure was 35 $mN \cdot m^{-1}$, the surface density in the outer deposited monolayer of DOPC was 0.88 ± 0.01 times that at the air-water interface. For DOPE the same factor was 0.90 ± 0.01. This expansion upon deposition again suggests that the deposited monolayer was in the fluid phase. We constructed isotherms for monolayers of each of the lipids at the air-water interface. In monolayers spread from organic solution there is always a significant incertitude in the absolute value of the area per molecule due to evaporation of the solvent. We do not report these isotherms here for that reason.

Homogeneity of mixed lipid bilayers

Are the mixed monolayers of DOPE-DOPC homogeneous? The distance resolution of the surface forces apparatus is sufficient to clearly resolve the difference in thickness between monolayers of DOPE and DOPC (see Fig. 1). The FECOs produced between surfaces bearing mixed monolayers were smooth and showed no sign of bilayer heterogeneity at the limit of resolution.

There is however the complication that the different repulsion between the two species could lead to demixing when the force between the surfaces is large at very close approach. At a given separation, the force per molecule is greater for DOPC than for DOPE (see Fig. 1) and so at sufficiently large interbilayer force, DOPC molecules might be expected to diffuse away from the contact zone, leaving a surface monolayer rich in DOPE. This problem has been analysed in detail by Bryant and Wolfe (1989) but we can give here an order of magnitude calculation. The energy per molecule due to an exponential repulsion between planes with characteristic length λ is $P a \lambda$ where P is the force per unit area and a the area per molecule, and other interactions have been neglected. Equilibrium demixing becomes substantial when the energy difference for the two molecules is comparable with the thermal energy kT, and so is important for pressures P of order $kT/a\lambda$, which for typical values is about 40 MPa. As the pressures reported here are rather smaller, such a local

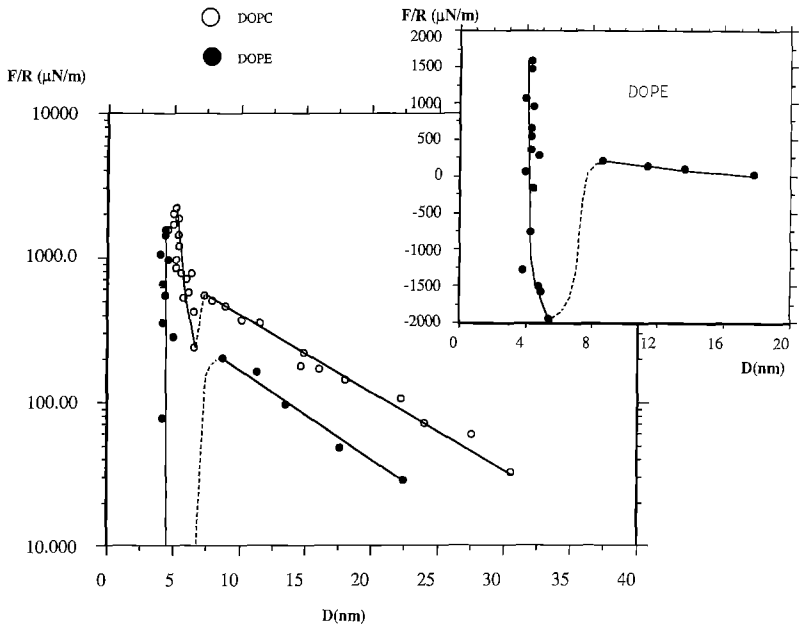

Fig. 1. Forces between two bilayers of DOPE (filled points) and DOPC (open points) across aqueous 1 mol · m^{-3} NaCl solution. The reference distance $D=0$ is that between two mica surfaces coated with one DOPE monolayer. The water thickness between the two phospholipid bilayers can be estimated from the distance D by subtracting approximately 4 nm. The attractive minimum for DOPE is shown in the insert. Solid lines are guides for the eye, and dashed lines indicate the forces and distances at which the system is out of equilibrium

change in composition is expected to be only a small effect.

A related question concerns the way in which bilayers may deform due to their interaction at close approach. When bilayers are pressed together in the experiments reported here[4], the energy of interaction increases among the molecules in the relatively small region of close approach. One might therefore expect molecules to diffuse in the plane of the bilayer, away from the region of close approach, and thus lower the total energy of the system. To obtain an approximate upper estimate to the extent of this effect, suppose that the lipids in the region of close approach equilibrate with those in the regions of large separation and negligible interaction. The former have a potential energy of interaction of about $P a \lambda$ (see above). The work to transfer a lipid of area a between two regions of a monolayer differing in lateral pressure by $\Delta\pi$ is $a\Delta\pi$. Neglecting differences in composition (discussed above), equilibrium requires $P a \lambda = a \Delta \pi$. The proportional change in molecular area $\Delta a/a = -\Delta\pi/k_A$ where k_A is the area elastic modulus of the monolayer, and so for small Δa,

$$\Delta a/a = P\lambda/k_A \,. \tag{1}$$

Taking k_A as 100 mN · m^{-1} and λ as 0.3 nm, the pressure required for a one percent change in a is about 30 MPa. The effect should therefore be small in the experiments reported here.

Forces

When two crossed cylinders are brought close together so that the separation d is much less than their radius R, the local geometry near the point of least separation represents that of a sphere with radius R near a plane. The force $F(d)$ for this geometry is simply related (Derjaguin's approximation) to the energy of interaction per unit area $U(d)$ between two parallel planes separated by d (a simpler but impractical geometry) thus: $F(d)/R = 2\pi U(d)$. For this reason the force curves presented have $F(d)/R$ as the ordinate.

The reference distance $D=0$ used in this paper is the contact between two mica surfaces both coated with one DOPE monolayer. The water thickness between the two phospholipid bilayers can be estimated from the distances shown in the force/distance profiles by subtracting approximately 4 nm (the thickness of two lipid monolayers).

The force between surfaces as a function of distance was qualitatively similar for all cases studied: there was a weak exponential repulsion at separations of up to 30–40 nm, and a weak attraction (a secondary minimum) at about 5 nm (DOPE) and 6 nm (DOPC). The magnitude of the attraction was larger for DOPE than for DOPC. Figure 1 shows a typical force distance curve for DOPC and another for DOPE.

In the region of exponential repulsion, the decay length was in all cases equal to the calculated Debye length of the aqueous solution (see Fig. 1). We interpret this as the force due to an electric double layer (Israelachvili and Adams 1978). The magnitude corresponded to a surface potential of 25 ± 5 mV for all the studied areas. This corresponds to approximately one charge per 90 nm^2 (deduced from Grahame's equation; see for example Israelachvili 1985). Marra (1985) has reported the forces of interaction between bilayers of saturated PC and PE, but reports that there are no double layer forces in the presence of monovalent cations. We can only speculate how the difference between saturated and unsaturated might lead to a difference in effective surface charge. Perhaps the unsaturated lipids are deposited at rather lower surface densities and that this allows surface charges (including adsorbed H^+ and OH^- ions) to remain on the

[4] Note however that the lateral constraint on these membranes is very different from that in lamellar phases where the membranes are brought together by dehydration and considerable compressive lateral stresses and strains may result (Lis et al. 1982; Wolfe 1987)

mica, or to migrate through it when the surface is immersed in solution. The saturated lipids form more tightly packed layers on the mica, and might exclude such surface charges. Charged impurities in the outer monolayer could in principle be responsible if 0.7% of the lipids were chemically changed to produce a dissociating form, but this is inconsistent with our chromatography results (see later). Further, Marra (1985) did not observe any impurity with the saturated lipids he purchased from the same company (Avanti Polar Lipids).

At distances of less than 5 nm (DOPE) and 6 nm (DOPC), the repulsion increased steeply with decreasing separation. This strong force at close approach is the hydration-steric force. Figure 1 shows that, at any given separation, the short range repulsion between DOPC bilayers is greater than that between DOPE bilayers in agreement with the results of Marra (1985). The separation in the figure is that between the mica surfaces coated with one monolayer of DOPE. The attraction minimum is much larger for DOPE (-2 mN · m) than for DOPC (± 0.2 mN · m^{-1}) which indicates smaller hydration-steric forces for DOPE than for DOPC. The short-range repulsion begins at a separation 0.8 nm greater for DOPC than for DOPE. This may be interpreted as the result of a larger hydration-steric repulsion for DOPC, or of the putative larger size of the higher DOPC headgroup. The difference between these interpretations is largely semantic.

When no fusion occurred, and after separation of the surfaces, subsequent force measurements were completely reproducible.

Fusion

When a sufficiently large, constant force was applied, the distance between the silvered surfaces reduced by about 4 nm over a period of 2 to 6 s. The process was similar to that reported by Horn (1984): a very small region on the fringes at the position of closest approach moved suddenly by an amount corresponding to 4 nm and then expanded to form a straight fringe corresponding to flat, parallel, circular regions with radii of typically 30 μm. The separation between the silvered surfaces in this state was typically 4 nm greater than between the silvered surfaces on two bare micas brought into contact. Following Horn, we interpret this as the fusion of two bilayers into one, proceeding from one point and spreading out to produce a single bilayer between the mica surfaces over a macroscopic area. We henceforth refer to this state as fusion (see footnote 1). In this state the surfaces adhered strongly and could be separated by a force of the order of 1 mN. This is not fully consistent with the adhesion of two fully hydrophobic surfaces in pure water, but suggests that on separation the single remaining bilayer separated to leave on each mica a monolayer plus some of the lipid that was squeezed out on fusion.

After fusion and separation of the surfaces, the forces between the regions that had fused were not reproducible. Repulsive forces of tens or hundreds of μN were measured at separations of tens of nm. Sufficiently large forces could overcome these irreproducible repulsions and bring the surfaces into close approach. These force-distance curves sometimes exhibited almost horizontal regions. This behaviour may be interpreted as the force-distance curve for the deformation of debris remaining after the fusion-separation cycle. After fusion had been produced at one point, other zones of the mica surfaces where the bilayers were not damaged could still be used for a limited number of further measurements.

The forces required to produce fusion were large (F of order 4 mN). When the mica surfaces of the apparatus are subjected to large pressures, they deform and the relation between F/R and the interaction energy of planar surfaces no longer obtains. At very large pressures the deformed surfaces form circular flat regions. Neglecting the force applied in the small area near the perimeter of this region and the weak forces exerted by the surfaces far from contact, the pressure acting on the region of close approach is just the force divided by the area of this circle. The pressures at which fusion occurred were about 2 MPa. There were no significant differences among the pressures required to fuse bilayers of pure DOPC, pure DOPE and of mixtures of the two.

This observation is relevant to any explanation of the reported effect of PE in enhancing the fusion of unconstrained bilayers. Our results indicate that such fusion enhancement cannot simply be related to the weaker repulsion at short distance between bilayers containing that lipid. In an alternative explanation, the fusion of unconstrained bilayers may involve local fluctuations in the normal direction and, if so, the effect of PE on fusion may be related to a putative effect on such undulations. In our experiments the movement of the bilayers in the normal direction is constrained by the mica substrate. Thus the lack of an effect of PE on fusion in our experiments is consistent with the latter explanation.

Chemical changes in unsaturated lipids

The results reported above were for experiments in which considerable care was taken to avoid oxidation of the lipids in chloroform solution. Solutions in chloroform were made up on the day of the experiment, the gas phase above the solution was rinsed with nitrogen, and the solution was kept in a sealed, light-tight container either in a freezer or in a container of (dry ice) CO_2. Thus the lipids were in contact with chloroform and air, and put at room temperature simultaneously for about half a minute while they were prepared, and for the time taken for most of the chloroform to evaporate during monolayer spreading (on the order of a minute). In other experiments we had used lipids in chloroform solutions which were kept in a freezer for periods of one to fifty days; these included experiments using lipids obtained from Sigma which were delivered in chloroform in sealed containers. With respect to inter-membrane forces, these "old" solutions gave results similar to those reported above. With respect to fusion, however, the results were different and depended on length of exposure to chloroform. The pressure required for fusion was always less than that required for fusion of

bilayers using freshly prepared solutions (correlation coefficient of F/R with respect to exposure to chloroform was -0.38). In contrast, however, the pressure required to produce fusion did not significantly decrease with time during experiments lasting up to two days during which the deposited bilayers were exposed to saline solution at room temperature and regularly exposed to intense illumination for the interferometry.

Chromatography

In order to find which transformation of the lipids had occurred, chromatographs of two lipid chloroform solutions were performed. One solution was stored for 8 months under nitrogen at $-5\,°C$; the other was prepared and used immediately.

It has been reported that auto-oxidation occurs in the unsaturated chains of phospholipids (Wu et al. 1982; Porter and Weenen 1981). This oxidation probably results from free radical peroxidation of the double bond in phospholipids (Barclay et al. 1987). In order to detect hydroperoxides possibly formed in DOPC and DOPE solutions, we adapted the thin layer chromatography method used by Barclay et al. for detecting hydroperoxides in DLPC and PLPC. Barclay et al. used for development the mixture hexane/2-propanol/acetone (992/4/4), and they used a spray of N,N-dimethyl-p-phenylene diamine dihydrochlorure (DMPDADH) (1.5 g in methanol/water/acetic acid (128/25/1) for detection. As our lipids did not migrate in this solvent, we used $CHCl_3$/n-Hexane/Methanol/acetic acid/water (60/35/20/15/1.5) (V/V) (Serrano de la Cruz et al. 1988) using DMPDADH spray for hydroperoxides, and iodine vapor to reveal unoxidized lipids.

Analyses were conducted to compare old and fresh lipid solutions. Two independent sets of measurements produced the same results:

- there was no trace of hydroperoxides in either solution
- a slight spot at the solvent front (revealed with iodine vapor) was observed on the chromatograms of the old lipid solution (zero retention). No such spot was observed with the fresh lipid solution. Such spots appear generally with very hydrophobic molecular species like triglycerides or fatty acids (Bandi and Ansari 1988; Kolarovic and Traitler 1985).

The presence of fatty acids could be due to the occurrence of phospholipid hydrolysis producing lysolecithin. This kind of degradation was described by Hauser in 1971 with liposomes under ultrasonic irradiation. In order to search lysolecithin possibly present in our samples, HPLC was performed on 8 months old and fresh DOPC and DOPE solutions. The mobile phase was acetonitrile/methanol/phosphoric acid (99/3/0.3, V/V) with UV detection (203 nm). The chromatograms of the old solution and of the fresh one were identical. No peak of lysolecithin was present. There is none or less than 0.1% lysolecithin in our old solutions. The detection limit was obtained after calibration of a standard of lysolecithin (data not shown).

Barclay et al. (1987) report that the presence of oxidation products in unsaturated phospholipids promote the formation of non-lamellar structures, and from this one might expect them to affect the stability of bilayers. The effects noted by these authors, however, occur when the concentrations of oxidation products exceeded 10%: two orders of magnitude greater than the upper limit of concentrations in our experiments.

The degradation products noted above and reactions which occur in the presence of chloroform probably have little relevance to biology. Nevertheless, the observation that trace quantities of fatty acids or lysolecithin may promote fusion (perhaps by nucleation) at much lower pressures may be relevant to explanations of the mechanism of fusion.

Finally, we note that other researchers using lipid extracts from tissue have observed the generation of oxidation products when using procedures necessitating chloroform solutions (Bryant and Lynch, personal communication). Therefore these results should be regarded as a warning of the danger of artifacts that may arise in experiments designed to determine the influence on fusion of DOPE and/or of other unsaturated lipids, particularly those which, like DOPE, may form non-lamellar phases. In view of the sensitivity of fusion to such low concentrations of contaminants, considerable care should be taken to distinguish between the effects of unsaturated lipids and that of their oxidation products.

Conclusion

The conclusion from this work is that the pressure which must be applied for membrane fusion is similar for bilayers whose opposing faces are pure DOPC, pure DOPE or mixtures of the two. The significant difference is rather that these pressures are achieved at smaller separation for bilayers whose opposing faces contain DOPE.

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