MEASUREMENT OF THE LATERAL COMPRESSIBILITY OF SEVERAL PHOSPHOLIPID BILAYERS

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ABSTRACT Lateral compressibilities of bilayers in multilayer lattices are given for 10 phospholipid preparations: dilauryl-, dimyristoyl-, dipalmitoyl-, distearoyl-, and dioleoylphosphatidylcholine (PC); egg phosphatidylethanolamine (PE); as well as cholesterol-containing bilayers of dipalmitoyl PC or of egg PC. Bilayer deformability is highly nonlinear and does not permit description in terms of a simple modulus. The presence of cholesterol or C=C bonds (dioleoyl PC) increases deformability, but freezing of acyl chains does not cause dramatic stiffening of the bilayer. Lateral compression of dilauryl PC and dimyristoyl PC causes a transition from "melted" to "frozen" acyl chains above the normal transition temperatures. Our measurements do not correspond in any obvious way to lateral compressibilities in monolayers at the air-water interface.

INTRODUCTION

Phospholipid bilayers, which form a large part of the structure of biological membranes, have been used in a number of configurations as model cellular membranes. Only very recently, however, has an experimental technique been devised to measure the lateral compressibility of bilayers (1). The lateral packing, interaction, and compressibility of molecules in the plane of biological membranes influence many of their important properties. Mobility and segregation of membrane components, incorporation of proteins or drugs into membranes, nonselective or enzyme-mediated membrane permeability, gross curvature changes, fusion, and assembly of membranes have all been shown to be affected by molecular packing. Lateral compressibility is a measure of the fluctuations in lateral packing and should be of fundamental importance in many of these membrane processes.

The monolayer has been the traditional experimental system for investigating lateral pressure, and an enormous body of data has been collected on the properties of phospholipid monolayers. Although they often seem to show a thermal phase transition analogous to that seen in the bulk phospholipids in water, it has been found that the properties of phospholipid monolayers can depend on the solvent needed to spread the films (2), on whether the monolayer is equilibrated with or without bulk lipid in the subphase (3), and in the case of lipids at temperatures

below their bulk phase transition (3), on the history of the monolayer. Indeed, the shoulder in phospholipid π -A curves, so often used to infer a monolayer phase transition, actually disappears when the surface film is not spread from a solvent carrier (N. Gershfeld, personal communication and reference 3). In addition, recent evidence shows that lecithin can attain bilayer densities at the air-water surface (4).

Whereas the behavior of monolayers appears increasingly complex, it is clear that there is at least one obvious difference between the behavior of phospholipids at an air-water or oil-water interface and a phospholipid bilayer that separates aqueous compartments. Unrestrained monolayer films spread to indefinitely high areas per molecule; bilayers, and indeed membranes, unrestrained by external forces in excess water, spread only to a limited and relatively condensed state. Because of this difference in lateral stability and the above-noted complexities in monolayer behavior, it is important to determine, experimentally, the compressibility of bilayers themselves.

Unlike monolayers, bilayers do not lend themselves easily to compression experiments. Some studies have been made on the expansion properties of bilayers. The most rigorous are those reported recently by Kwok and Evans (5), who used direct sucking by pipette on solvent-free vesicular bilayers. Many studies involve electrocompression, i.e., thinning and area expansion of planar phospholipid bilayers that contain an appreciable (6, 7) or negligible (8) amount of solvent. The problems of solvent extrusion are serious in estimating bilayer deformability. Solvent-

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deprived bilayers give different results from those containing large amounts of solvent.

With a technique devised initially to measure the forces of interaction between bilayer membranes (1), we now report intrabilayer forces as well. The bilayers are compressed under a known external force from their fully expanded state in water. For egg lecithin we measured (1) the lateral pressure in the bilayer as the molecular area decreased by ~25%, which was analogous to measuring a π - A isotherm for a monolayer. In this paper we report similar measurements on 10 different neutral phospholipid bilayer systems including some with melted and some with frozen hydrocarbon chains as well as two that contain cholesterol. These studies show that (a) at least some phospholipids have bilayer lateral pressure-area isotherms that are qualitatively different from those of their corresponding monolayers; (b) on compression some bilayers undergo major phase transitions not seen in monolayer compression; (c) there are large differences in the compressibility of different bilayers, e.g., cholesterol at certain concentrations or double bonds in the hydrocarbon chains greatly enhance the compressibility of bilayers.

MATERIALS

Dilauroylphosphatidylcholine (DLPC), dimyristoyl PC (DMPC), dipalmitoyl PC (DPPC), distearoyl PC (DSPC), dioleoyl PC (DOPC) were obtained from Sigma Chemical Company (St. Louis, Mo.). In addition, DPPC was purchased from Serdary Research Laboratories, (London, Ontario) or synthesized by us using the method of Robles et al. (9). Egg phosphatidylethanolamine (Egg PE) was purchased from Nutfield Lipid Products (Surrey, England) and cholesterol from Calbiochem-Behring Corp. (San Diego, Calif.). All lipids were checked periodically for purity by thin-layer chromatography and stored under nitrogen at -18°C until used. Contamination was always $<1\%$. Mixtures of phospholipids with cholesterol were prepared by dissolving each lipid in chloroform, combining the solutions, and removing the solvent by rotary evaporation with final drying under vacuum. Dextran (T-2,000; 2,000,000 mol wt) was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Water, double-distilled in glass, was used for all lipid and dextran samples. Except for DPPC at 50°C and DMPC at 27°C , all measurements were made at 25°C .

METHODS

We have described in detail (1, 11) the technique of measuring the work of compressing the phospholipid bilayers and simultaneously measuring the decrease in molecular area. Very briefly the strategy is as follows. All these phospholipid systems in water form multilamellar structures made up of alternating layers of water and bimolecular phospholipid leaflets. X-ray diffraction of this one-dimensional lattice and a knowledge of the composition of the lamellar phase yields the bilayer thickness d_l , bilayer separation, d_w , and molecular area, A , available to each phospholipid molecule on the bilayer-water interface (11). The water in the lamellar phase is equilibrated with external water of known chemical potential, which is set by osmotic, hydrostatic, or vapor pressure. As the external chemical potential of the water is reduced, the lattice loses water and the lipid molecules become more closely packed in the bilayer, causing an increase in d_l and decrease in A . The measured chemical potential gives the internal pressure P in the lamellar phase.

The change in molecular free energy, g , with change in molecular area is

$$\partial g / \partial A = P(d_w/2) = F_{LP}(\text{dynes/cm}), \quad (1)$$

and gives the lateral pressure in the bilayer at each molecular area A . As noted in previous papers (1, 11) evaluations of $\partial g / \partial A$ at different areas for each lipid are in fact taken at different d_w as well as A . It is really a function of the two variables, $F_{LP} = F_{LP}(A; d_w)$ but will be plotted as an explicit function of A only.

Assuming the separability of F_{LP} from the interlamellar force F_R , and integrating $\partial g / \partial A$ vs. A numerically from the equilibrium area A_0 seen in excess bulk water, the energy E_c of compressing the bilayer can be estimated. To compare behavior of different materials, the ratio

$$C = - \frac{\Delta A / A_0}{F_{LP}} (\text{cm/dyne}) \quad (2)$$

is found for each bilayer. C gives the relative change in area for an applied lateral pressure, F_{LP} . [In terms of C , the modulus used in reference 1 is $2/(d_l C)$.]

RESULTS

Fig. 1 gives the lateral pressure vs. molecular area for the 10 different lipid systems in the low compression region. The lines through the data points are drawn by eye; no simple expression fits these curves qualitatively well enough to allow a best-fit line to be determined.

Instructive in comparing the lipids are the plots in Fig. 2, which give the lateral pressure that results when the molecular area is reduced from A_0 , where the lateral pressure $\partial g / \partial A$ is zero. Fig. 2a is for the synthetic lecithins under small compression; Fig. 2b, for the effect of cholesterol in dipalmitoylphosphatidylcholine bilayers, melted or frozen; and Fig. 2c, for those lipids which can undergo a large change in molecular area or lateral pressure without going through a phase change or transition. Comparisons are summarized in Table I, wherein are listed A_0 , F_{LP} for a $4\text{-}\text{\AA}^2$ change in molecular area, E_c^4 the work required to produce this change in area, and compressibility C . Estimates of compressibility are difficult near the equilibrium area A_0 because of the scatter in molecular area: they were determined graphically for $4\text{-}\text{\AA}^2$ changes in molecular area assuming linearity of $\partial g / \partial A$ vs. A near A_0 . By measuring

FIGURE 1 Lateral pressure $F_{LP}(A; d_w) = \partial g / \partial A$ vs. molecular cross-sectional area in 10 preparations. Areas for mixtures with cholesterol are normalized per phospholipid headgroup.

FIGURE 2 Data of Fig. 1 replotted as lateral pressures vs. extent of lateral compression ($A - A_0$) to compare behavior near A_0 . (a and b) are results for small lateral pressures. (c) shows high pressures encountered in large deformation. Open symbols designate melted hydrocarbon chains. Insets provide details of data near origin.

the area under the curves, we estimate the work E_c^{total} of compressing the bilayer membranes from A_0 to the minimum area found for each lipid. These works are also given in Table I; all are <1 kT /molecule (k , Boltzmann's constant; T , absolute temperature).

Many of these bilayers undergo a phase change or phase

TABLE I
SUMMARY OF COMPRESSIBILITY PARAMETERS

Lipid	A_0	F_{LP}^*	E_c^{total}	E_c^*	C
	$\text{\AA}^2$	dyne/cm	$\times 10^3$	$\times 10^5$	$\times 10^3$ cm/dyne
Disordered hydrocarbon chains					
DLPC	68.7	0.58	107	217	101
DMPC (27°C)	65.2	1.0	5	387	61
DPPC (50°C)	71.2	0.68	9	227	83
DOPC	82.0	0.05	156	26	975
DPPC:chol, 1:1	96.5	0.13	25	68	320
Egg PC	75.6	0.32	250	112	167
Egg PC:chol, 1:1	96.0	0.60	3	133	70
Egg PE	74.7	0.80	291	460	67
Crystalline hydrocarbon chains					
DPPC	52.3	1.4	58	421	55
DSPC	54.7	1.8	20	734	41
DPPC:chol, 8:1	54.0	0.22	26	57	325

A_0 , molecular area per phospholipid polar group of bilayer fully expanded in excess water; F_{LP}^* , lateral pressure developed by compression of molecular area from A_0 to $(A_0 - 4) \text{\AA}^2$; E_c^{total} , work of compressing molecular area from A_0 to the minimum area observed (in units of kT); E_c^* , work of compressing polar group area from A_0 to $(A_0 - 4) \text{\AA}^2$ (in units of kT); C , relative change in area divided by lateral pressure determined at $A_0 - 4 \text{\AA}^2$ using the curves of Fig. 1 and Eq. 2 as described in the text. All data at 25°C unless otherwise noted.

separation on compression beyond the point shown in Fig. 2. The resulting phases are not characterized in detail but are as follows: DLPC undergoes a phase change, at a molecular area of $56.0 \text{\AA}^2$, that results in the crystallization of its hydrocarbon chains. DSPC undergoes a restructuring of its already crystallized chains at a molecular area below $48.6 \text{\AA}^2$. We have reported elsewhere (12) how the lipids containing cholesterol show very clear phase separation; pure cholesterol separates from the 1:1 mole-ratio mixtures and two lamellar phases segregate in the case of DPPC:cholesterol 8:1 (12) at molecular areas of $79.8 \text{\AA}^2$ and $48.1 \text{\AA}^2$, respectively. Such separations are to be expected in view of the studies by Tajima and Gershfeld (13, 14), although we do not see them at 1:1 ratios in the unstressed state.

DISCUSSION

We defer to a separate study the use of our deformation measurements to test and improve various theories of bilayer mechanics and of the hydrocarbon chain transition in phospholipid bilayers. In the past, monolayer rather than bilayer surface pressure vs. area (π - A) curves have been used to infer the deformability of bilayers for testing against theoretical prediction. As discussed above, Gershfeld and co-workers (3, 4, 15) have already described the pitfalls in using monolayer data for such purposes. We now find that there is no easily apparent correspondence between monolayer π - A curves and our corresponding measurements of $\partial g/\partial A$ vs. A .

The points on Fig. 3a-c, are bilayer lateral pressure measurements $\partial g/\partial A = F_{LP}(A; d_w)$ for DOPC, DLPC, and DPPC. The lines are bilayer lateral pressures inferred from monolayer surface pressures using the prescription of

FIGURE 3 Comparison of measured lateral pressures with those inferred from monolayer data. The reported lateral pressure for the monolayer, π^0 , at the measured bilayer equilibrium area, A_0 , was subtracted from the monolayer pressures. 3(a) DOPC: curve data from reference 16. (b) DLPC: Curve 1 data from reference 17; curve 2, (18; deduced from curves for di10 PC and di14 PC); curve 3 from M. C. Phillips (1978), personal communication. (c) DPPC Curve 1 data from reference 19; 2, 20; 3, 21; 4, 2 where curve 4a was for DPPC spread with chloroform, 4b with 1:1 chloroform:hexane, and 4c with 1:4 ethanol:petroleum ether. Curve 5, data from reference 22; 6, 23 with 6a for "purified" lipid and 6b for "unpurified". Curve 7 data from reference 3 where 7a is for a film spread from crystal and 7b for a solvent-spread film; 8 from 24.

Evans and Waugh (25) that the two pressures should differ by a constant "surface tension," subtracted from monolayer measurements, to bring the net surface pressure to zero at the measured unstressed bilayer equilibrium area A_0 .

It is distressingly apparent that (a) there are internal inconsistencies between monolayer data from different laboratories or from different preparations in the same

laboratory and (b) that in no obvious way do the derived curves agree with directly measured bilayer lateral pressures. Furthermore, monolayer data give no indication of the phase-transition discontinuity so sharply apparent in the bilayer measurements. Differences in monolayer and multilayer systems clearly make difficult any attempts to draw strong parallels between them.

Under lateral stress, both DLPC (see Fig. 3b) and

DMPC (not shown) undergo a gel to liquid-crystal phase transition at 25°C. This is far above the -1.3°C melting temperature of unstressed DLPC (13). A sudden change in cross-sectional area occurs at this transition under a lateral pressure of 12 dynes/cm².

We are so far unable to devise a mathematical form to summarize accurately our lateral pressure vs. area data in the way we were able to summarize bilayer repulsion forces with an exponential decay (1, 11). Attempts to fit the $\partial g/\partial A$ vs. A data with quadratic equations consistently give force minima at less than the observed equilibrium area.

We are as yet unable to reconcile these compressibility measurements with the extensibility measurements of Kwok and Evans (5) made on single layer vesicles. In those measurements, egg PC bilayer bubbles, expanded up to 3% before bursting, showed an expansive modulus $(\partial g/\partial A)/(\Delta A/A_0)$ of 140 dyne/cm vs. our 6 dyne/cm (translating our C from Table I for egg PC when $\Delta A = 4 \text{ \AA}^2$.) An apparently soft initial extensibility was explained as being due to bending rather than stretching the bilayer (5). It is possible that the mechanical properties of isolated bilayers in fact differ from those of bilayers in multilayer arrays.

One should note that if the interlamellar force $F_R = \partial g/\partial d_w$ decreases with decreasing area A then $F_{LP} = \partial g/\partial A$ measured along the equilibrium (d_w, A) line will be a softer function than would be obtained if one could measure $\partial g/\partial A$ for lateral compression at constant d_w .

As a means of codifying the data we have computed the energies of compression shown in Table I. Elsewhere (26, 27), compressibility moduli have been used to estimate the fluctuations in lateral pressure in monolayer systems. The "compressibility" defined in Eq. 2 is used only as a guide for comparison among the several lipids and is evaluated for area changes $\Delta A = 4 \text{ \AA}^2$ only. The deformation energies in the Table are all less than kT . They indicate that very large fluctuations in molecular area and bilayer thickness can be expected for all these bilayers (except perhaps for the restraining influence of the multilayer regime).

Considering only the larger differences shown by Fig. 2 and Table I the following comparisons among the different bilayers can be made.

Crystalline vs. Disordered Hydrocarbon Chains

DPPC frozen and melted (Fig. 2b) provides the most direct comparison of the effect of melting the hydrocarbon chains on bilayer compressibility. Although melting increases compressibility, and in general the compressibility of melted bilayers is greater than that of frozen (Fig. 2a and Table I), it is surprising that melting the chains has a smaller effect on compressibility than other factors discussed below. (In contrast, bilayer extensibility is decreased by an order of magnitude when chains freeze

[5].) It appears to us that the tilt of the solid hydrocarbon chains changes relatively easily compared with the work of compressing polar groups. A suggestion of such ease can be inferred from the recent observation that 1 uranyl ion bound per 10 DPPC molecules can completely remove the 30° tilt usually seen in bilayers of frozen DPPC (26).

Effect of Polar Group

The differences between egg PC and egg PE shown in Fig. 2c suggest a significant dependence of the compressibility of bilayers on the nature of the polar group. The data for DOPC and DPPC per cholesterol in Figs. 2a and b indicate that the more hydrated PC bilayers are also the more compressible.

Effect of Structural Perturbants

Indeed the most compressible bilayers are DOPC, DPPC:chol 8:1 and 1:1. Fig. 2b shows that cholesterol produces a large increase in the compressibility of DPPC bilayers whether the hydrocarbon chains remain crystalline (DPPC:chol, 8:1) or whether they are disordered (DPPC:chol, 1:1). Fig. 2a shows that disorder introduced into the hydrocarbon chain layer by the presence of double bonds rather than by melting saturated chains results in a greatly enhanced compressibility. Both of these results suggest that major structural perturbants, like cholesterol or permanent kinks in the chains, introduce packing imperfections that endow bilayers of saturated lipids with the capacity to suffer large decreases in area without commensurate increases in lateral pressure. However, the fact that cholesterol has the opposite effect in egg PC bilayers suggests that cholesterol perturbs much less those bilayers that have very heterogeneous populations of chains.

Other comparisons might be made, but we expect at least three interactions to contribute to the lateral pressure (29): (a) repulsion between the head groups, (b) repulsion in the hydrocarbon region, and (c) interfacial tension between the hydrophobic and hydrophilic layers. (a) and (c) probably depend only weakly on the hydrocarbon chains and more on the kind, hydration and conformation of the polar groups. (b) is likely to be sensitive to chain length and conformation. This is the contribution usually emphasized in theories of bilayer phase transitions. (b) appears to be profoundly affected by structural perturbants: intrinsic proteins could play such a role in membrane compressibility.

The nonlinear variation of $\partial g/\partial A$ indicates that many such kinds of interaction may simultaneously or successively dominate the lateral pressure as a bilayer is deformed.

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