Structural Changes in Lipid Bilayers and Biological Membranes Caused by Hydrostatic Pressure

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ABSTRACT: By use of neutron diffraction, the structural parameters of oriented multilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine with deuteriocarbon chains/cholesterol (molar ratio 70:30), multilamellar lipid vesicles composed of pure lipids and lipid/cholesterol mixtures, and crystalline purple membrane patches from *Halobacterium halobium* have been measured at pressures up to 2 kbar. Pressurization of the oriented 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine/cholesterol multilayers results in an in-plane compression with the mean deuteriocarbon chain spacing of 4.44 Å obtained under ambient conditions decreasing by 3–7% at 1.9 kbar. The thickness for this bilayer increases by $\simeq$1.5 Å, but the net bilayer volume decreases and the isothermal compressibility is estimated to be in the range (−0.1 to −0.6) × 10^{-4}/bar at 19.0 °C. The *d* spacings for multilamellar vesicles composed of lipids in the liquid crystalline state and lipid/cholesterol mixtures increase linearly as a function of pressure, suggesting that these bilayers are also compressed in the membrane plane. For 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine and 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine MLVs in the gel state, the *d* spacing decreases with pressure. For 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine, the hexagonally packed chains are anisotropically compressed in the bilayer plane, resulting in a pseudohexagonal chain packing at 1.9 kbar. The bilayer compressibility is (−0.4 or −0.5) × 10^{-4}/bar depending on whether the chain tilt increases with pressure or terminal methyl groups of apposing lipid monolayers approach each other. Crystalline purple membrane patches composed of protein and lipid in the volume ratio 2:1 are compressed in the plane, with the unit cell area decreasing by $\simeq$4% on pressurization to 2.0 kbar.

The effects of hydrostatic pressure on many membrane-mediated processes are well documented [for a review see Macdonald (1984)]. By Le Chatelier's principle, pressurization should result in a net volume decrease of the system and for cell membranes the volume decrease may occur by simple bulk compression of the lipid bilayer or proteins and/or changes of chemical equilibria in solution such as the separation of ion pairs at the membrane–water interface or the dissociation of multimeric protein assemblies (Weber & Drickamer, 1983).

Pressure-induced membrane structural changes may affect membrane-related phenomena, but, so far, structural measurements of membranes at high pressure have been lacking. We have measured the changes in structural parameters of fully hydrated lipid bilayers, lipid bilayers containing cholesterol, and purple membrane patches from *Halobacterium halobium* as a function of hydrostatic pressure. Previously, by using neutron diffraction, we have investigated the temperature–pressure phase diagrams for several single-component phospholipid bilayers up to pressures of 2 kbar (Braganza & Worcester, 1986).

Materials and Methods

Materials. DMPC,[1] DPPC, and DSPC were purchased from Fluka AG ($\simeq$99% pure). EPC, DMPC with deuteriocarbon chains, and cholesterol were obtained from Lipid Products, Lipid Specialities Inc. (Boston, MA), and Sigma Chemical Co. (St. Louis, MO), respectively. All products were used without further purification. The deuterium oxide (D_2O) used was of 99.8% isotopic purity.

Sample Preparation. Oriented lipid multilayers and multilamellar vesicles were prepared as described before (Braganza & Worcester, 1986). MLV dispersions contained typically 20 mg of lipid/mL of D_2O buffer (5 mM Tris, 5 mM KCl, 125 mM NaCl, pD 7.5). Purple membrane patches from *H. halobium* were provided by Dr. G. Zaccai (Institut Laue-Langevin). Isolation procedures were carried out as described by Zaccai and Gilmore (1979). The membrane patches ($\simeq$100 mg) were dried down onto several quartz slides, and the slide assembly was used for the diffraction experiments.

Experimental Methods. The cylindrical pressure cell fabricated from a "null" scattering titanium–zirconium alloy and rated for 2 kbar has been described previously (Neilson et al., 1979). Pressure was generated by means of a hand pump, being recorded on a Budenberg gauge and transmitted to the cell filled with D_2O via a fluid line of light machine oil. The cell was placed in an aluminum block thermostated by circulating water, and a NiCr/NiAl thermocouple positioned between the cell and aluminum block monitored the temperature. Quartz slides with oriented lipid multilayers for lamellar and chain diffraction measurements were mounted vertically and horizontally in the cell, respectively. MLV preparations were pipetted directly into the cell, and a Teflon insert was used to isolate the stirred preparations from the aqueous–oil interface.

The neutron diffraction experiments were carried out on diffractometer D16 at the high-flux reactor of the Institut

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[1] Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine; EPC, egg phosphatidylcholine; ESR, electron spin resonance; MLVs, multilamellar lipid vesicles.

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Laue-Langevin, Grenoble, France. The diffractometer, which has been described previously (Braganza & Worcester, 1986), uses neutrons of wavelength 4.52 Å and has a Q range of 0.03–2.30 Å^{-1} [$Q = (4\pi \sin \theta)/\lambda$]. Collimation is provided by a pair of slits mounted on an optical bench, and a ^{3}He gas wire multidetector 16 cm wide and 8 cm high counts diffracted neutrons. The neutron beam size was typically 8 mm wide by 20 mm high, and a sample to detector distance of 100 cm was used.

Bragg d spacings were calculated by using $n\lambda = 2d \sin \theta_n$ where n refers to the diffraction order, λ is the wavelength, and θ_n is the Bragg angle of the nth order. For oriented multilayers the coherent scattering amplitude density $\rho(x)$ across the unit cell was calculated according to

$$\rho(x) = \sum \pm |F_n| \cos (2\pi n x/d)$$

where $|F_n|$ is the structure factor modulus of the nth diffraction order. The structure factor moduli were calculated from the diffraction intensities, I_n, according to $|F_n| = [[\sin (2\theta_n)]I_n]^{1/2}$, where $\sin (2\theta_n)$ is the Lorentz factor (Arndt & Willis, 1966). The mosaic spread of the oriented multilayers was $\simeq 1°$ so that more than 95% of the total diffraction intensity was accepted by the two-dimensional detector.

The isothermal compressibility is defined as $\beta = -1/V\,(dV/dP)_T$ and is simply the fractional volume change of the bilayer dV/V per bar of pressure. The quantity β was calculated from the change in length and cross-sectional area occupied per lipid molecule as a function of pressure, obtained from the diffraction data.

Results

Measurements for multilayers of DMPC with deuteriocarbon chains/cholesterol (molar ratio 70:30) in D_2O were made with the ω–2θ mode of diffraction (Arndt & Willis, 1966). The sample was well oriented with a mosaic spread of $\simeq 1°$ at 19.0 °C and ambient pressure, giving six diffraction orders. The relative phases in D_2O for oriented multilayers of similar lipid/cholesterol mixtures have been determined previously (Worcester, unpublished data)[2] by "swelling" experiments and by H_2O–D_2O exchange (Worcester, 1976). The scattering density profile shown in Figure 1a was calculated from these phases (−, −, +, +, +, −) together with the structure factors obtained from the present data for multilayers immersed in D_2O in the pressure cell at ambient pressure and 19.0 °C. The regions of high scattering density are due to the deuteriocarbon chains and D_2O between bilayers, with minima at the bilayer center and for the protonated lipid head groups. Pressurization to 1.9 kbar results in a small d spacing increase from 54.5 to 56.5 Å. Since the structure factor magnitudes are much greater than 0 and the continuous Fourier transform of the unit cell is sampled only over a small distance in reciprocal space, the relative phases for the ambient- and high-pressure structures are essentially the same. The calculated profile at 1.9 kbar is shown in Figure 1b and illustrates that most of the 2-Å increase in d spacing on pressurization is accounted for by a thickness increase of the bilayer, with possibly an additional small increase in the D_2O region. The glycerol backbone and phosphatidylcholine moiety of the lipid molecules cannot be resolved separately in the Fourier profiles so the distance between head group minima was taken to be a measure of the bilayer thickness. Changes of head group

[2] Data collected on the small-angle scattering diffractometer at the Harwell reactor PLUTO, Atomic Energy Research Establishment, Harwell, Berkshire, U.K.

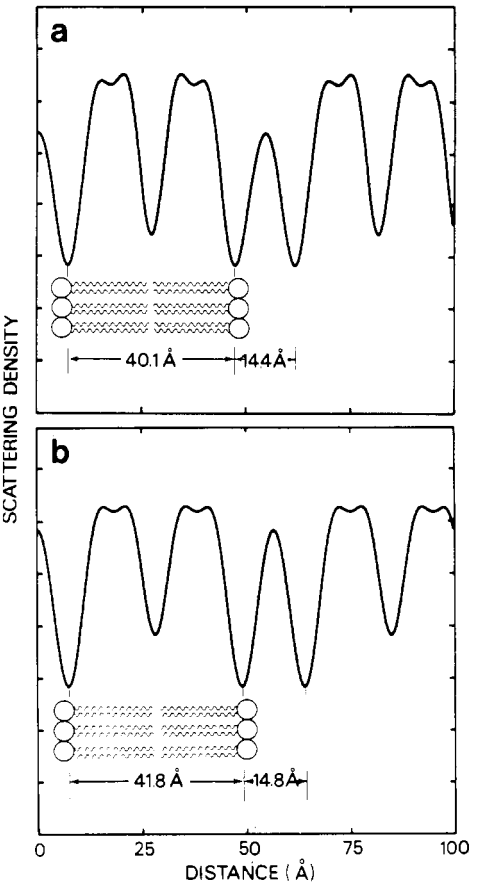

FIGURE 1: Scattering density profiles for oriented multilayers of DMPC with deuteriocarbon chains/cholesterol (molar ratio 70:30) in D_2O at 19.0 °C and (a) ambient pressure, d = 54.5 Å, and (b) 1.9 kbar, d = 56.5 Å. Schematic drawings of the bilayer are shown beneath each profile. The values for the bilayer thickness and aqueous space are given to the first decimal place and reflect the precision of the ruler measurement only. The phased structure factors used in the Fourier syntheses (origin in the aqueous space) were (a) −25.1, −19.2, +41.2, +9.8, +14.9, −9.0 and (b) −22.3, −12.8, +40.2, +13.9, +13.7, −6.9. Diffraction patterns were recorded over a period of 65 min. Errors in the structure factors due to counting statistics were less than ±0.5% for the prominent peaks and $\simeq \pm 2.0\%$ for the weak diffraction peaks. Some asymmetry was observed in the peak shapes and was most evident for the strong third order, which showed a shoulder on the lower scattering angle side of the peak corresponding to a second d spacing of approximately 63 Å. This is most probably due to the presence of multilamellar vesicles that form spontaneously in excess aqueous phase and have a repeat unit of about 65 Å (see Figure 3).

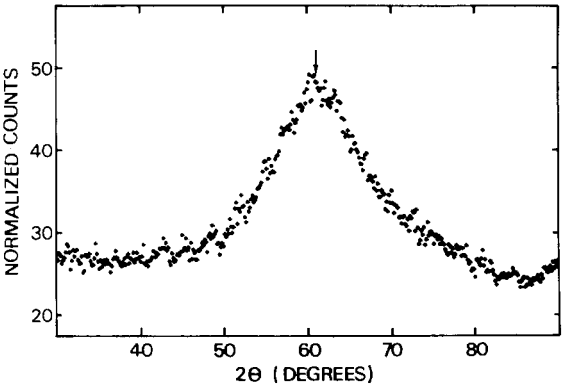

FIGURE 2: Wide-angle diffraction pattern from oriented multilayers of DMPC with deuteriocarbon chains/cholesterol (molar ratio 70:30) at 19 °C, at ambient pressure and $\simeq 50\%$ relative humidity outside the pressure cell. The mean reciprocal chain spacing is at (4.44 Å)$^{-1}$ with a full width at half-maximum range of values from (4.10 Å)$^{-1}$ to (4.90 Å)$^{-1}$.

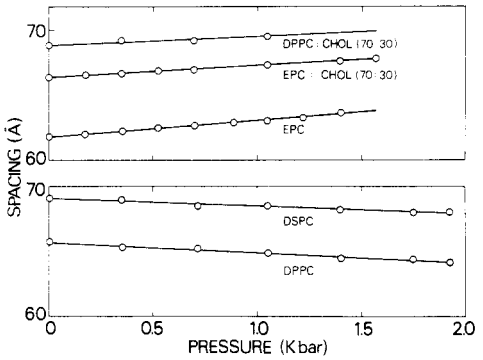

FIGURE 3: First-order Bragg spacing as a function of pressure for multilamellar vesicles in D_2O pD 7.5 buffer solution of EPC, EPC/cholesterol (molar ratio 70:30), DPPC/cholesterol (molar ratio 70:30), DPPC, and DSPC at 25 °C. The spacings are accurate to ±0.2 Å.

conformation with pressure cannot, however, be ruled out.

The wide-angle chain diffraction from DMPC with deuteriocarbon chains/cholesterol (molar ratio 70:30) bilayers at ambient pressure and ≃50% relative humidity is shown in Figure 2. The broad diffraction peak, indicating that chain packing is disrupted by cholesterol molelcules, has a full width at half-maximum of 12.0° in 2θ. This corresponds to a range of reciprocal chain spacing values from $(4.10\ Å)^{-1}$ to $(4.90\ Å)^{-1}$ with the peak center at $(4.44\ Å)^{-1}$. A poor chain diffraction peak height to background ratio was obtained with the sample immersed in D_2O in the pressure cell. This is due to poor contrast and to D_2O scattering, which for $\lambda = 4.52$ Å rises sharply at scattering angles around 50° and peaks at 68° (Neilson et al., 1979). Under these experimental conditions, the chain diffraction peak was observed on a positively sloping background as a low-intensity shoulder spanning 15° in 2θ with a peak height to background ratio of 0.05. The mean reciprocal spacing was estimated to be between $(4.45\ Å)^{-1}$ and $(4.55\ Å)^{-1}$ at ambient pressure and to lie between $(4.20\ Å)^{-1}$ and $(4.30\ Å)^{-1}$ at 2.0 kbar, giving a percentage decrease in mean reciprocal chain spacing of 3–7%. Assuming a isotropic compression in the bilayer plane and no change in the type of packing, this represents a cross-sectional area decrease of approximately 6–14% per deuteriocarbon chain from 1 bar to 1.9 kbar (since the area is proportional to the square of the chain spacing). Taking into account the thickness increase of the bilayer with pressure, a compressibility in the range -0.1×10^{-4}/bar to -0.6×10^{-4}/bar is obtained.

Figure 3 shows the *d* spacing as a function of pressure for MLVs of EPC, EPC/cholesterol (molar ratio 70:30), DPPC/cholesterol (molar ratio 70:30), DPPC, and DSPC in D_2O pD 7.5 buffer solution. The *d* spacings for the cholesterol-containing lipid preparations increase linearly with pressure, most of the change being accounted for by the thickening of the bilayer (see Figure 2) as a result of the in-plane compression of hydrocarbon chains. The largest in-plane compression is observed for egg phosphatidylcholine MLVs where the *d* spacing increases by 2.5 ± 0.2 Å over a 2.0-kbar pressure range. At room temperature the heterogeneous chains are in a liquid crystalline or disordered state so that presumably the loose-chain packing is responsible for the high lateral compressibility. A smaller *d* spacing increase of 2.0 ± 0.2 Å is observed for EPC/cholesterol (molar ratio 70:30) MLVs between ambient pressure and 2.0 kbar, apparently due to the ordering effect of cholesterol on the hydrocarbon chains. The *d* spacing at ambient pressure also increases to 66.3 from 61.8 Å for EPC vesicles and is due mainly to the increase in bilayer thickness as a consequence

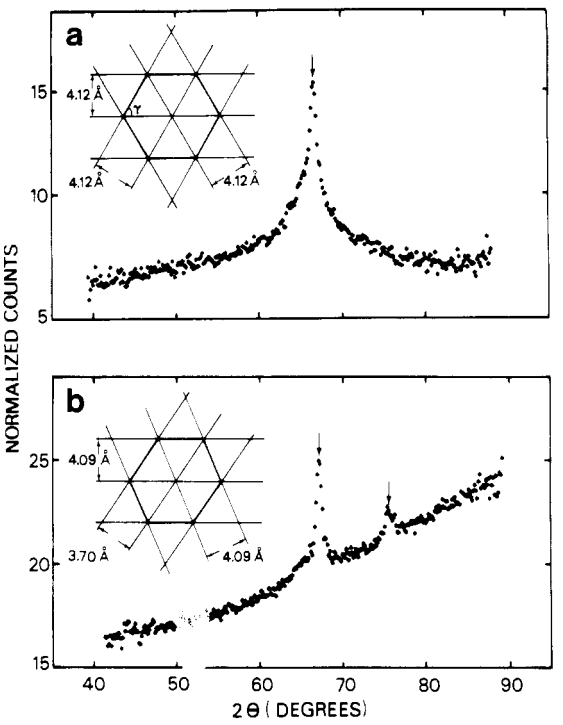

FIGURE 4: Wide-angle diffraction pattern from oriented multilayers of DPPC with deuteriocarbon chains (a) at 19 °C, ambient pressure, and ≃50% relative humidity outside the pressure cell with the peak at $(4.12\ Å)^{-1}$ and (b) at 19 °C and 1.9 kbar with peaks at $(4.09\ Å)^{-1}$ and $(3.70\ Å)^{-1}$. The suggested chain-packing lattices that give the diffraction patterns are shown in the insets.

of chain ordering by cholesterol (Rand & Luzzatti, 1968).

For DPPC and DSPC MLVs at 25 °C, the pressure response is different. The bilayers are in the gel state and the *d* spacing decreases by approximately 1.5 Å over 2.0 kbar. DPPC/cholesterol (molar ratio 70:30) MLVs, however, show a liquid crystalline type pressure response with the *d* spacing increasing as a function of pressure and illustrate that the addition of cholesterol to gel state bilayers perturbs the ordered chain packing. The ambient-pressure *d* spacing also increases from 65.7 Å for DPPC MLV's at 25 °C to 68.8 Å for DPPC preparations with 30 mol % cholesterol. This result is in good agreement with those of Lis et al. (1982) who derive values for the bilayer thickness and bilayer separation that indicate that although high levels of cholesterol cause a significant decrease in gel state DPPC bilayer thickness, a larger increase occurs in bilayer separation as a result of the spreading of polar lipid head groups.

The wide-angle diffraction pattern obtained for DPPC with deuteriocarbon chains at ambient pressure and ≃50% relative humidity at 19.0 °C is shown in Figure 4a. The sharp, nearly symmetrical peak at $(4.12\ Å)^{-1}$ is characteristically obtained for DPPC bilayers with a water content lower than 10% and is indicative of hexagonally packed untilted chains (Tardieu et al., 1973). Each chain has six nearest neighbors at 4.76 Å, and the cross-sectional area per chain was calculated to be 19.60 Å^2 from $2d_h^2 3^{-1/2}$ where d_h is the chain spacing. The diffraction pattern obtained for the same sample in the pressure cell at 1.9 kbar and 19.0 °C is shown in Figure 4b. The $(4.12\ Å)^{-1}$ peak observed at ambient pressure is shifted to a smaller reciprocal spacing of $(4.09\ Å)^{-1}$, and a second diffraction peak is evident at $(3.70\ Å)^{-1}$. This diffraction pattern suggests that the hexagonally packed chains at ambient pressure are compressed anisotropically in the plane, resulting in a pseudo-hexagonal chain lattice at 1.9 kbar. For hexagonal packing, the angle between the **a** and **b** unit vectors (defined as the γ

angle) of the two-dimensional lattice is 60.0° (see inset, Figure 4a). The peaks at $(4.09\ Å)^{-1}$ and $(3.70\ Å)^{-1}$ in Figure 4b can only occur for γ angles of 63.11°, each chain having four nearest neighbors at 4.59 Å and two at 4.15 Å, or for γ = 56.5°, with each chain having four nearest neighbors at 4.44 Å and two at 4.90 Å. However, since the diffraction intensity of the $(4.09\ Å)^{-1}$ peak is approximately twice that of the $(3.70\ Å)^{-1}$ peak the γ angle must be 56.5° because the chains are then packed such that there are two diffraction planes at 4.09-Å separation and one at 3.70-Å separation (see inset, Figure 4b). For γ = 56.5°, the cross-sectional area occupied per chain is 18.15 $Å^2$ (cf, 19.60 $Å^2$ at ambient pressure). Gel-state bilayers of DPPC and DSPC have tilted, ordered hydrocarbon chains (Tardieu et al., 1973; Janiak et al., 1976) so that the decrease in *d* spacing with pressure is due either to compression perpendicular to the bilayer surface such that the terminal methyl groups of opposing monolayers approach each other or to an increase in the chain tilt angle. A value for the compressibility of -0.4×10^{-4}/bar is calculated if chain tilt increases with pressure and -0.5×10^{-4}/bar if compression occurs perpendicular to the bilayer surface.

Diffraction measurements from the in-plane hexagonal lattice of crystalline purple membrane fragments were carried out as described by Zaccai and Gilmore (1979). In the D_2O-filled pressure cell and as a function of time, membrane fragments became detached from the quartz slides, and so good counting statistics were obtained only for the strong (1,1) reflection at ambient pressure and 2 kbar (data not shown). The in-plane lattice parameter decreased from 62.4 Å at ambient pressure to 61.1 Å at 2.0 kbar and corresponds to a 4% decrease in unit cell area. Because of sample loss and poor orientation in D_2O, lamellar diffraction was weak and so any membrane thickness changes with pressure could not be monitored.

Discussion

The wide-angle diffraction measurements on oriented multilayers of DMPC with deuteriocarbon chains/cholesterol (molar ratio 70:30) indicate that the bilayer is compressed laterally with the cross-sectional area occupied per deuteriocarbon chain decreasing by 6–14% on pressurization to 1.9 kbar. This result is in good agreement with ESR (Chin et al., 1976) and fluorescence polarization measurements (Chong & Cossins, 1983; Chong & Weber, 1983) which demonstrate that increasing pressure causes progressive ordering of probe molecules located in the hydrocarbon region of fluid lipid bilayers and biological membranes. The scattering density profiles across the lamellar structure provide direct evidence that the bilayer thickness increases from $\simeq$40.0 Å at ambient pressure to $\simeq$41.5 Å at 1.9 kbar as a consequence of compression in the bilayer plane. However, the net volume of the bilayer decreases and the isothermal compressibility is estimated to be in the range $(-0.1$ to $-0.6) \times 10^{-4}$/bar. This range of compressibilities is typical of polymers and organic solids [$\beta \simeq (-0.2$ to $-0.5) \times 10^{-4}$/bar] but lower than the compressibilities of organic liquids such as hexadecane, methanol, and pentanol ($\beta \simeq -1.0 \times 10^{-4}$/bar).

Measurements of *d* spacings as a function of pressure for multilamellar lipid vesicles suggest that the bilayer lateral compressibility is proportional to the amount of hydrocarbon chain disorder. Of the lipid vesicles studied, EPC bilayers, whose chains are disordered at room temperature, show the largest increase in *d* spacing over a 2.0-kbar range. The addition of 30 mol % cholesterol to EPC bilayers caused a reduction in the *d* spacing vs. pressure slope (and by implication a reduction in the lateral compressibility) due to the ordering effect of cholesterol on "fluid" hydrocarbon chains. Cholesterol apparently has the opposite effect on gel state bilayers and perturbs the ordered chain packing.

Normal compressibilities, in the sense of bilayer thickness and cross-sectional area occupied per lipid chain decreasing with pressure, were only observed for single-component lipid bilayers in the gel state. DPPC and DSPC multilamellar vesicles gave *d* spacing decreases of 1.5 Å over a 2.0-kbar pressure range. The wide-angle diffraction pattern from DPPC with deuteriocarbon chains indicates that the chains, which are packed in a hexagonal lattice with six nearest neighbors at 4.76 Å under ambient conditions, are compressed anisotropically in the plane, resulting in a pseudohexagonal lattice at 1.9 kbar with four nearest neighbors at 4.44 Å and two at 4.90 Å. Steric hindrance to isotropic in-plane compression may arise from the rectangular cross-section of an all-trans hydrocarbon chain and also from the lipid glycerol backbone to which two hydrocarbon chains are attached via ester linkages. A similar chain diffraction pattern to that shown in Figure 4b has been observed for DMPC bilayers at −8 °C (Janiak et al., 1976), illustrating that increasing pressure has the same effect as lowering temperature.

Previously, Stamatoff et al. (1979) have used X-ray diffraction to measure the *d* spacing as a function of pressure up to 3.0 kbar for DPPC containing 13% by weight water and obtained a spacing vs. pressure behavior essentially similar to that observed in the present study for multilamellar vesicles at a lipid concentration of 20 mg/mL D_2O; in the liquid crystalline phase the *d* spacing increases and in the gel phase decreases as a function of pressure.

The measurements on purple membrane fragments demonstrate that, as for lipid/cholesterol bilayers, natural membranes compress in the plane with pressure. Purple membrane fragments are composed of the protein bacteriorhodopsin and lipid in the volume ratio 2:1 with the protein arranged in a hexagonal two-dimensional lattice in the membrane plane [for a review see Stoeckenius and Bogomolni (1982)]. Each unit cell has 3 bacteriorhodopsin molecules and 36 lipid molecules arranged in a bilayer with the ratio of the unit cell area occupied by protein to lipid being appoximately 2:1 (Zaccai & Gilmore, 1979). The diffraction data were insufficient to allow any differences in the lateral compresibilities between the protein and lipid regions to be observed. Proteins, however, have intrinsic compressibilities of $\simeq 10^{-5}$/bar (Gavish et al., 1983), which is lower than the estimate of compressibility for lipid/cholesterol bilayers [$(-0.1$ to $-0.6) \times 10^{-4}$/bar]. Assuming, therefore, that most of the 4% decrease in unit cell area between ambient pressure and 2.0 kbar takes place due to an isotropic lateral compression of the lipid region, a decrease in the cross-sectional area occupied per lipid molecule of $\simeq$12% is obtained and is comparable to the 6–14% decrease in cross-sectional area observed per deuteriocarbon chain over the same pressure range for lipid/cholesterol bilayers.

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Registry No. DMPC, 18194-24-6; DPPC, 63-89-8; DSPC, 816-94-4; cholesterol, 57-88-5.

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Factors Affecting Surface Expression of Glycolipids: Influence of Lipid Environment and Ceramide Composition on Antibody Recognition of Cerebroside Sulfate in Liposomes†

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ABSTRACT: The reactivity of the acidic glycolipid cerebroside sulfate (CBS) with antibody was studied as a function of its lipid environment in vesicles and of its ceramide composition. The lipid environment was varied by using phosphatidylcholine of varying chain length with cholesterol in a phosphatidylcholine:cholesterol:cerebroside sulfate molar ratio to glycolipid of 1:0.75:0.1. The ceramide structure of CBS was varied by using synthetic forms containing palmitic acid, lignoceric acid, or the corresponding α-hydroxy fatty acids. Reactivity with antibody was determined by measuring complement-mediated lysis of the vesicles containing a spin-label marker, tempocholine chloride. The data were analyzed by a theoretical model which gives relative values for the dissociation constant and concentration of antibodies within the antiserum which are able to bind to the glycolipid. If the phosphatidylcholine chain length was increased, increasing the bilayer thickness, only a small population of high-affinity antibodies were able to bind to cerebroside sulfate, suggesting decreased surface exposure of the glycosyl head group. A larger population of lower affinity antibodies were able to bind to it in a shorter chain length phosphatidylcholine environment. However, if the chain length of the cerebroside sulfate was increased, it could be recognized by more antibodies of lower affinity than the short chain length form, suggesting that an increase in chain length of the glycolipid increased surface exposure. Hydroxylation of the fatty acid inhibited antibody binding; only a smaller population of higher affinity antibodies was able to bind to the hydroxy fatty acid forms. This suggests that hydroxylation may decrease the surface exposure or alter the head-group conformation so that most of the antibodies in this polyclonal antiserum are unable to bind. Thus, changes in the lipid composition of plasma membranes may affect the surface exposure of glycolipids or the ability of receptors to bind to them and thus alter cell recognition.

Glycolipids are present in the plasma membrane of a great number of different types of cells. Their location and the existence of a multiplicity of different carbohydrate structures suggest that they play more than a structural role in the membrane. Indeed, glycolipids have been implicated in cell development, differentiation, and carcinogenic transformation (Hakomori, 1981). The ability of glycolipids to act as haptens and elicit immune responses suggests the possibility that they may also play a role in autoimmune diseases such as multiple sclerosis (Webb & Fazakerley, 1984; Offner et al., 1981). The expression or exposure of glycolipids on the cell surface may be altered in malignant tissue and different cells in cases where there is no change in glycolipid content (Young et al., 1981; Kannagi et al., 1982; Nudelman et al., 1982). It is therefore

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