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Surface ripples cause the large fluid spaces between gel phase bilayers containing small amounts of cholesterol

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Previous studies have found that small concentrations of cholesterol, or several other molecules such as benzene and asialoganglioside, dramatically increase the fluid separation between gel phase phosphatidylcholine bilayers. These observations can not be explained in terms of changes in the repulsive and attractive pressures known to exist between flat gel phase bilayer surfaces. We show here that the increase in fluid space occurs as a consequence of cholesterol inducing large periodic ripples in the plane of the bilayer. The analysis of Mortensen et al. (Biochim. Biophys. Acta 945, 221–245) indicates that the sides of the ripples primarily contain gel phase phosphatidylcholine, whereas the apices are enriched in cholesterol and are liquid-crystalline. We argue that the large fluid spaces can be explained by steric repulsion between adjacent bilayers caused both by thermally induced accordion-like motions of these ripples and defects in the ripple organization. In addition, ripples potentially can decrease van der Waals attraction and change hydration repulsion between bilayers.

Introduction

Since cholesterol is an important component of most mammalian plasma membranes, there have been many experimental and theoretical investigations of the effects that cholesterol has on the properties of cell membranes and lipid bilayers [1]. In particular, the attractive and repulsive interactions between apposing bilayer surfaces have been analyzed as a function of cholesterol content [2–4]. In the liquid-crystalline phase, the equilibrium spacing between bilayers containing cholesterol can be understood in terms of a balance between the repulsive hydration, steric, and undulation pressures and the attractive van der Waals pressure [4]. However, an intriguing observation of four laboratories [2,5–7] is that the incorporation of small concentrations of cholesterol into gel phase phosphatidylcholine (PC) bilayers dramatically increases the lamellar repeat period between adjacent bilayers. Since the incorporation of cholesterol has only small effects on bilayer thickness, these data imply that small concentrations of cholesterol cause large increases in the fluid spacing (d_f) between adjacent bilayers [2,5]. This large increase in d_f is not observed in liquid-crystalline phase PC bilayers containing cholesterol [3,4,6,7], and, we argue here, can not be readily explained in terms of changes in the pressures acting between the surfaces of *planar*, electrically neutral gel phase bilayers.

This report presents evidence that the increase in d_f at low cholesterol concentrations is a consequence of cholesterol inducing large, periodic ripples in the plane of the bilayer [7–10]. We argue that these ripples can cause steric hindrance to the close approach of adjacent bilayers, and also modify the van der Waals and hydration pressures.

Results

Figs. 1A and 1B show lamellar repeat periods for dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) bilayers, respectively, as a function of cholesterol content. These X-ray data were obtained by Hui and He [6] and Rand et al. [2] at temperatures below both the pre-transition and main transition temperatures of the pure PC bilayers. For both DMPC (Fig. 1A) and DPPC (Fig. 1B), the repeat period abruptly increases with the addition of small (2 to 10%) concentrations of cholesterol and then gradu-

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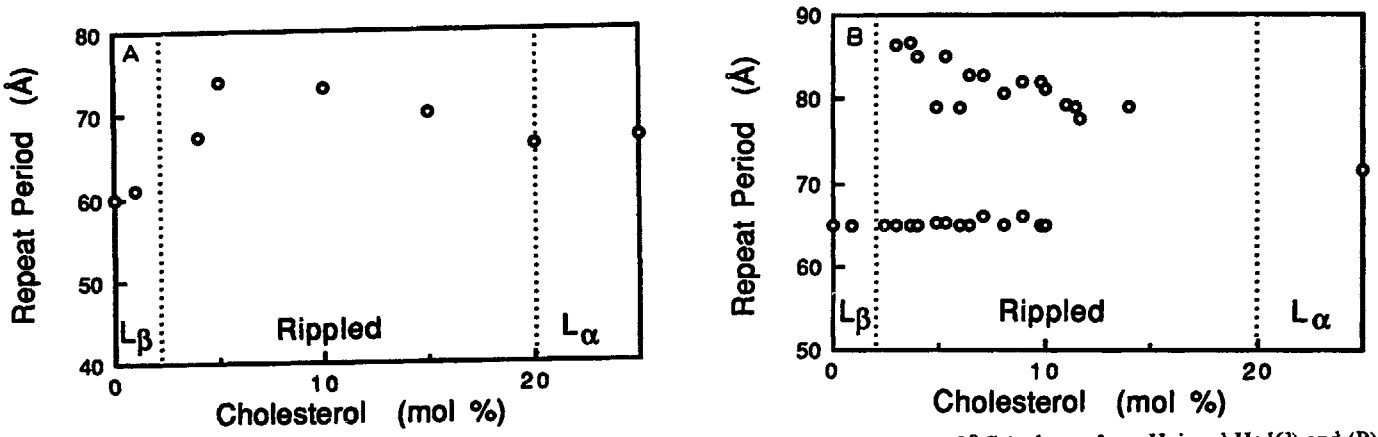

Fig. 1. Lamellar X-ray repeat periods as a function of cholesterol content for (A) DMPC bilayers at 5°C (redrawn from Hui and He [6]) and (B) DPPC bilayers at 23–26°C (redrawn from Rand et al. [2]). Also shown as vertical dashed lines are the approximate positions of phase boundaries between the planar gel (L_β or $L_{\beta'}$) phase, a phase containing ripples in its surface, and the planar liquid-crystalline (L_α) phase. The positions of these phase boundaries are taken from Copeland and McConnell [8], Lentz et al. [9], Knoll et al. [12], and Mortensen et al. [7].

ally decreases with further addition of cholesterol. (As shown in Fig. 1B, for DPPC bilayers Rand et al. [2] observed two lamellar phases for cholesterol contents between 3 and 11 mol%, one with the same repeat period as pure DPPC and the second with a much larger repeat period. For DPPC with similar cholesterol contents, Ladbrooke et al. [5] observed only the phase with the larger repeat period.) For fully hydrated equimolar DPPC/cholesterol bilayers, the repeat period is about 65 Å, or the same as pure DPPC [2]. Since the bilayer thicknesses of PC and PC/cholesterol bilayers are nearly the same [2,11], the data presented in Figs. 1A and 1B indicate that small amounts of cholesterol increase d_f for gel phase DMPC or DPPC bilayers by about 15 to 25 Å. That is, the relatively small increase in bilayer thickness caused by cholesterol reducing hydrocarbon chain tilt [11] cannot, by itself, explain the large increases in lamellar repeat period observed with the addition of small amounts of cholesterol (Figs. 1A and 1B). Ladbrooke et al. [5] also concluded that the addition of small quantities of cholesterol increases the fluid spacing between bilayers. In contrast, above the main transition temperature the lamellar repeat period of DMPC does not appreciably increase with small concentrations of cholesterol and is nearly independent of cholesterol content [6].

Also shown in Figs. 1A and 1B are phase boundaries for DMPC/cholesterol and DPPC/cholesterol bilayers as determined by separate freeze-fracture [8,9,12] and small-angle neutron diffraction [7,12] studies. The incorporation of 2 to 3 mol% cholesterol into planar gel phase ($L_{\beta'}$) PC bilayers introduces large ripples in the bilayer surface. In DMPC/cholesterol bilayers at low cholesterol concentrations (< 8 mol%), Knoll et al. [12] and Mortensen et al. [7] found ripples whose wavelength is a function of temperature. For example, at 2 mol% cholesterol, Mortensen et al. [7] observe a wavelength of 360 Å at 4°C which decreases to 220 Å at 14.5°C, and Knoll et al. [12] report a smaller wavelength of about 140 Å at 17°C. Other workers [8,9] have also observed this second class of smaller ripples. At higher cholesterol concentrations (up to 24 mol%), Knoll et al. [12] noted that "...the ripple texture is completely distorted...; that is the vesicle surface exhibits elongated smooth patches separated by damlike protrusions." At cholesterol concentrations of greater than 20 to 24 mol%, the ripples disappear and the bilayer becomes flat [8,12].

Thus, there is a strong correlation between the induction of ripples in the plane of the bilayer and the observed increases in lamellar repeat period and fluid separation. At temperatures below the pre-transition temperature, unusually large values of repeat period are observed for DMPC/cholesterol and DPPC/cholesterol bilayers at cholesterol concentrations between about 2 and 20 mol% where the bilayer surface contains ripples (Figs. 1A and 1B). The repeat periods are significantly smaller at lower (< 2 mol%) or higher (> 20 mol%) cholesterol concentrations where the bilayers are flat or planar. Moreover, the incorporation of cholesterol at any concentration does not markedly increase the repeat period for DMPC bilayers at temperatures above the main transition temperature [6], where the bilayer surface is flat [9].

Direct evidence that ripples can indeed increase d_f comes from two additional sources. First, d_f is about 4 Å wider for the rippled ($P_{\beta'}$) phase than for the planar ($L_{\beta'}$) gel phase of DPPC [13]. For the $P_{\beta'}$ phase the ripple wavelength is about 120 Å and estimates for the ripple height or amplitude (as measured from trough-to-crest of the ripple) range from 10 Å [14] to 50 Å [15]. Second, the addition of benzene to DPPC bilayers induces ripples in the bilayer surface and, as shown by X-ray diffraction analysis, increases both the lamellar repeat period and the fluid separation between bilayers by an average of about 20 Å [16]. In DPPC liposomes

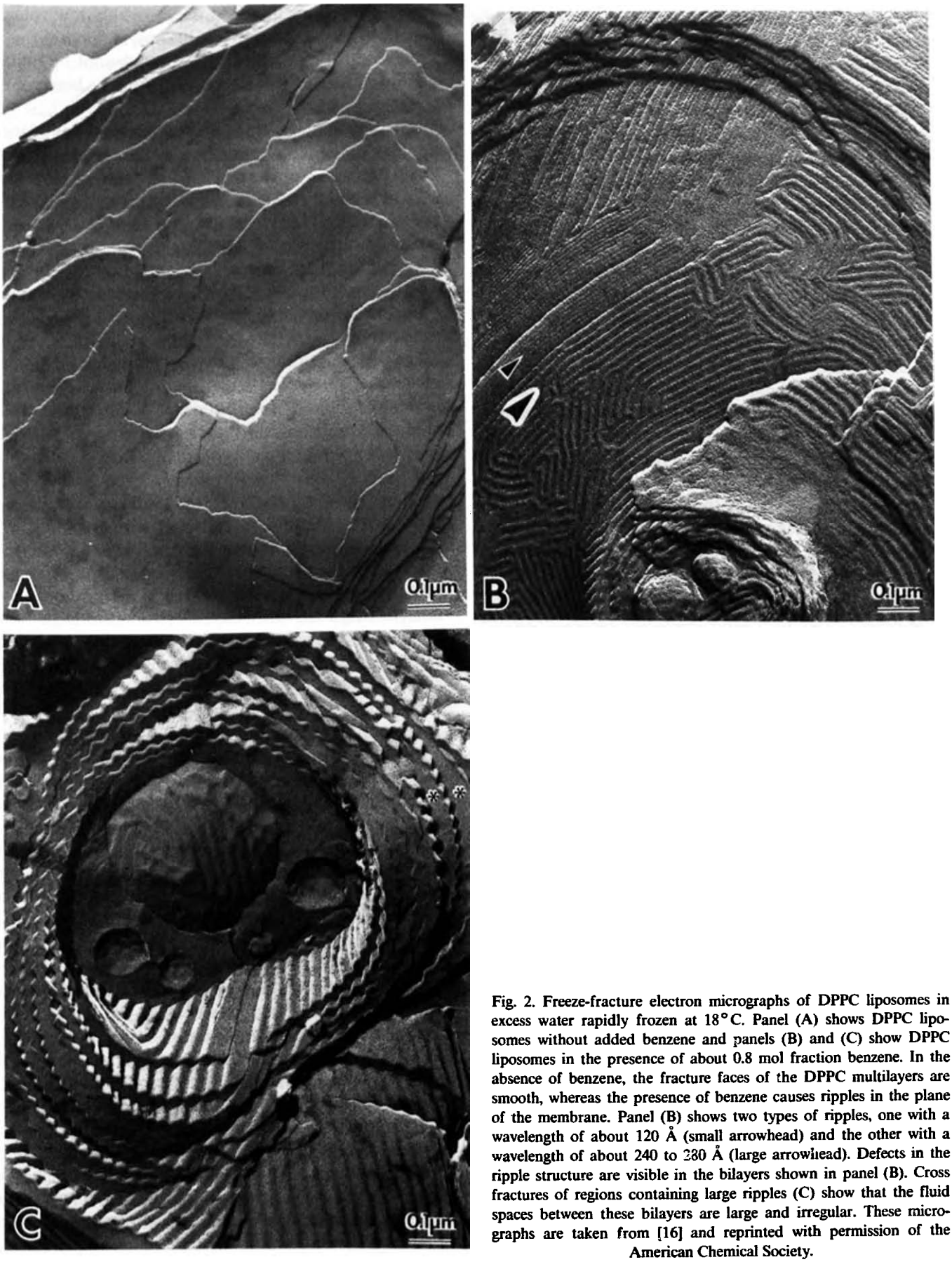

Fig. 2. Freeze-fracture electron micrographs of DPPC liposomes in excess water rapidly frozen at 18°C. Panel (A) shows DPPC liposomes without added benzene and panels (B) and (C) show DPPC liposomes in the presence of about 0.8 mol fraction benzene. In the absence of benzene, the fracture faces of the DPPC multilayers are smooth, whereas the presence of benzene causes ripples in the plane of the membrane. Panel (B) shows two types of ripples, one with a wavelength of about 120 Å (small arrowhead) and the other with a wavelength of about 240 to 280 Å (large arrowhead). Defects in the ripple structure are visible in the bilayers shown in panel (B). Cross fractures of regions containing large ripples (C) show that the fluid spaces between these bilayers are large and irregular. These micrographs are taken from [16] and reprinted with permission of the American Chemical Society.

containing benzene there are two types of ripples present, fine ripples with a wavelength of about 120–130 Å, similar to those observed in the $P_{\beta'}$ phase, and larger, deeper ripples with a wavelength of 230–360 Å (Fig. 2B). The large ripples are similar in wavelength to those observed by Mortensen et al. [7] and Knoll et al. [12] for DMPC/cholesterol bilayers at 4° C. Moreover, freeze-fracture images of bilayer cross fractures provide a direct visualization of the fluid spaces between adjacent bilayers. Fig. 2C shows cross fractures of a region with particularly large ripple wavelengths (about 360 Å) with a trough-to-crest amplitude of about 140 Å [16]. The fluid spaces between the bilayers containing these very large ripples are quite variable and, in certain areas, as large as 200 Å (asterisks in Fig. 2C).

A variety of molecules induce a rippled structure when added to gel phase ($L_{\beta'}$) PC bilayers. In addition to cholesterol [8,12] and benzene [16], phosphatidylserine [17], alamethicin [18], glycophorin [19], photosynthetic reaction center protein [20], and asialoganglioside GM_1 [21] all induce ripples in PC bilayers. The latter example is particularly relevant to this study, since asialoganglioside GM_1 has been directly visualized at the peaks and valleys of the ripples [21]. Moreover, in freeze-fracture images of rippled DMPC/asialoganglioside bilayers, the fluid spaces appear to be extremely large [21]. Since asialoganglioside GM_1 is not charged, electrostatic repulsion can not explain the large fluid separations. Here also the ripples themselves might be involved in increasing the fluid separation between bilayers.

Discussion

The large fluid spaces between DPPC or DMPC bilayers containing about 3 mol% cholesterol can not be explained by changes in the balance of attractive and repulsive pressures that are known to exist between gel phase *planar* bilayers. For such bilayers the repulsive undulation pressure, due to thermally induced undulative displacements of bilayers from planar configurations [22,23], is very small [24]. The only pressures operating between electrically neutral, planar gel phase PC bilayers are the repulsive hydration pressure, P_h, and the attractive van der Waals pressure, P_v [2]. Thus, in order for there to be an increase in d_f between *planar* gel phase PC bilayers, there would have to be either an increase in P_h and/or a decrease in P_v. However, the addition of cholesterol would be expected to decrease P_h, since liquid-crystalline bilayers containing 0.2 to 0.5 mol fraction cholesterol have smaller hydration pressures than do gel phase bilayers [4], and slightly increase P_v, since the addition of cholesterol into bilayers increases the Hamaker constant of the van der Waals pressure [25]. Moreover, if one considered the equilibrium fluid separation solely in terms of a balance between the repulsive hydration and attractive van der Waals pressures, this large value of d_f could be obtained only if one assumed unreasonably low values of the Hamaker constant [2,3,16]. Thus, it appears that the increase in d_f caused by cholesterol cannot be rationalized in terms of changes in P_h or P_v between *flat* surfaces.

There are at least three possible explanations for how ripples in the bilayer surface could increase d_f: (1) fluctuations in ripple amplitude or defects in the ripple structure could result in a repulsive steric pressure between adjacent bilayers, (2) van der Waals attraction could be diminished if the ripples in adjacent bilayers were not in phase, and (3) the magnitude of the repulsive hydration pressure could be increased by modulations in the bilayer surface.

First consider the possibility that flexible ripples cause steric hindrance to close approach of adjacent bilayers. The rippled structure for PC/cholesterol bilayers has some unusual properties. Mortensen et al. [7] analyzed neutron diffraction patterns of DMPC/cholesterol bilayers and concluded that cholesterol (at < 20 mol%) is localized in the peaks and valleys of the ripples. They suggest that the peaks and valleys are fluidized by the incorporation of cholesterol, whereas the flat regions between the peaks and valleys are composed of gel phase PC. Support for this model comes from the work of Ipsen et al. [10], who analyzed nuclear magnetic resonance, electron paramagnetic resonance, and calorimetry data to show that the incorporation of about 6 mol% cholesterol into DPPC bilayers at 25° C converts the $L_{\beta'}$ phase into a phase containing both solid and liquid components.

Thus, DPPC bilayers containing small amounts of cholesterol are thought to form a pleated surface with solid sides and fluid hinges. Such a rippled bilayer can display thermally driven accordion-like motions with fluctuations in ripple width and amplitude. If these fluctuations in width or amplitude are comparable to d_f of gel phase planar bilayers, then appreciable steric repulsion between adjacent rippled bilayers will occur and cause d_f to be greater for rippled bilayers than for flat bilayers. To approximate the size of the fluctuations in amplitude and width, we consider the image of the cross fractures of the large ripples in DPPC/benzene bilayers (Fig. 2C) and assume that the fluctuations in ripple size as a function of time can be approximated by the variation in ripple size for many ripples observed at one particular moment. For the ripples in Fig. 2C, we measure the width to be 356 ± 79 Å (mean ± S.D., $N = 76$ measurements) and the peak-to-valley amplitude to be 135 ± 18 Å ($N = 20$). Note that because of the image geometry, the ripple amplitude can only be measured accurately in instances where the fracture plane is nearly perpendicular to the surface of the bilayer, and therefore the amplitudes of some of the ripples with the

largest widths could not be measured. Thus, in the case of the very large ripples in DPPC/benzene bilayers (Fig. 2C), deviations in ripple width and ripple amplitude are much greater than the measured $d_f \approx 12$ Å for gel ($L_{\beta'}$) phase PC bilayers [26]. This calculation indicates that accordion-like motion of these large ripples can lead to steric hindrance between adjacent bilayers. We do not have corresponding measurements for the variations in the ripple width and amplitude for the 120 to 140 Å wavelength ripples of $P_{\beta'}$ phase of saturated PCs or the 220 to 360 Å wavelength ripples observed in DMPC/cholesterol bilayers [12]. However, based on the above measurements and the similarity in ripple wavelength for DPPC/benzene [16] and DMPC/cholesterol [7] bilayers, it seems reasonable to assume that the deviations in ripple width and amplitude for the DMPC/cholesterol bilayers are at least comparable to the measured $d_f \approx 12$ Å for gel ($L_{\beta'}$) phase PC bilayers. Consistent with this idea is the observation that for DMPC/cholesterol bilayers the neutron scattering reflection associated with the rippled structure is quite broad [7], indicating that there is a relatively wide range of ripple widths in the specimen.

The steric pressure described above is similar to the undulation pressure in fluid bilayers [22,23] in the sense that the ripples will increase the configurational entropy contribution to the interaction free energy of the system. This will result in an enhanced repulsive pressure for rippled bilayers as compared to flat bilayers. However, the steric pressure between rippled bilayers should have a different range and magnitude compared to the undulation force for fluid planar bilayers, since the modes of motions for these systems are quite different. If the ripples do indeed increase the configurational entropy of the system, then one would expect that in order for adjacent rippled bilayers to come close together the ripples would have to be removed. In fact, it has been observed that removal of a portion of the interbilayer water converts the rippled $P_{\beta'}$ phase into a planar gel phase [27–29].

For the steric pressure caused by ripples to increase the equilibrium fluid spacing, the energy to remove the ripples should be comparable in magnitude to the work to remove water from between planar bilayers. From the membrane tension versus projected lipid area data presented in Fig. 3 of Needham and Evans [30], we calculate that it takes approximately $8.7 \cdot 10^{-2}$ erg/cm^2 to remove the ripples from the $P_{\beta'}$ phase of fully hydrated DMPC bilayers. For either DMPC or DPPC, the $P_{\beta'}$ phase is also converted to a planar gel phase by the removal of about 4 water molecules per lipid molecule [27]. The work to remove 4 water molecules per lipid from the $L_{\beta'}$ phase of DPPC can be calculated from our published value for P_h for DPPC [26]. Assuming that the area per lipid molecule remains constant, we calculate that it would take about $4.4 \cdot 10^{-2}$ erg/cm^2 to remove 4 water molecules from the $L_{\beta'}$ phase of DPPC when the only repulsive pressure acting is the hydration pressure. These calculations indicate that the energy to remove the ripples from the $P_{\beta'}$ phase is of the same order of magnitude as the energy to remove 4 water molecules per lipid from between flat $L_{\beta'}$ phase bilayers. Thus, we argue that, near full hydration, the steric pressure caused by these ripples is comparable in magnitude to the hydration pressure, and would therefore be large enough to increase d_f.

The volume of fluid perturbed by these accordion-like excursions, and therefore the magnitude of this steric hindrance, should depend not only on the variation in amplitude, but also on the uniformity of the ripples. Freeze-fracture electron micrographs [8,9,12,16,31] reveal a variety of defects in the ripple structure, such as variations in the amplitude, orientation, and wavelength of the ripples, as well as edge dislocations and disclinations [32,33]. Such defects would be expected to increase d_f for rippled bilayers relative to planar bilayers, since they must prevent ripples in adjacent bilayers from lining up in phase. When the ripples from adjacent bilayers are not in phase and when the peak-to-valley amplitudes of the ripples are larger than the average d_f, there will be steric interactions between ripple peaks from adjacent bilayers. In fact, the ripple amplitudes of 10 Å to 50 Å for the $P_{\beta'}$ phase [14,15] and about 140 Å for DPPC/benzene bilayers [16] are comparable to, or larger than, the measured value of $d_f \approx 12$ Å for flat, gel phase PC bilayers [26].

The increase in fluid separation caused by fluctuations and defects in the ripple structure could also lead to another factor which would further increase d_f. As discussed above, these geometrical factors cause a decrease in the periodic alignment of the apposing bilayers and therefore prevent adjacent bilayers from packing together as closely as planar bilayers. Thus, the ripple structure prevents adjacent bilayers from maximizing the attractive van der Waals pressure, P_v. For regions containing large fluid spaces, retardation effects [34] would further reduce P_v between adjacent bilayers. In neutral bilayers, such as PCs, P_v is the only attractive pressure that limits swelling between bilayers. Therefore, even small reductions in P_v can lead to relatively large increases in d_f since P_v decreases as $d_f^{-1/3}$ [35].

Another interbilayer interaction which might be different between rippled surfaces than between flat surfaces is the repulsive hydration pressure. Should ripples increase P_h, as indicated by the recent theoretical analysis of Goldstein and Leibler [36], then d_f should be larger for rippled bilayers than for flat bilayers. X-ray diffraction experiments of Wack and Webb [37] on the $P_{\beta'}$ phase of various saturated phosphatidylcholine bilayers suggest a coupling of the ripples and fluid spaces between bilayers through intermembrane hydration interactions.

The hydration pressure, for a number of bilayer systems, has been found empirically to have the functional form $P_h = P_o \exp(-d_f/\lambda)$ where λ is the decay length which is 1 to 2 Å for PC or PC/cholesterol bilayers [4,26,38]. Therefore, increases in either P_o or λ would increase the equilibrium value of d_f. Although there are many theoretical models for P_h, it is commonly accepted that, for planar bilayers, the polarization of water decays exponentially from each bilayer surface reaching its minimum in the geometric center of the fluid space [38–41]. For flat bilayers of uniform composition, water molecules will have the same magnitude of polarization, on average, along any plane parallel to the bilayer surface. However, when ripples are present the polarization profile will be more complicated and depend on the ratio of the base to the height of the ripples. Moreover, for the rippled phase of DMPC/cholesterol, the cholesterol is localized primarily in the peaks and valleys of the ripple [7]. For these bilayers there will be two polarization profiles or hydration pressures, each with different values of P_o and λ [4], one corresponding to the gel DPPC phases strips and the other to the cholesterol-containing peaks and valleys. Water molecules located between these two regions will have some intermediate orientation. It is difficult to predict a priori whether such perturbations would enhance or diminish P_h.

In summary, we conclude that the large increase in fluid space between gel phase bilayers induced by small concentrations of cholesterol arises from the presence of ripples with fluid vertices and rigid sides. Ripples might increase d_f by several mechanisms, including a decrease in van der Waals attraction, an increase in the repulsive hydration pressure, or, most importantly, steric repulsion caused by fluctuations and defects in the ripple structure. For any of these possible mechanisms, large and irregular ripples (found in PC/cholesterol mixtures) would be expected to produce larger increases in d_f than small, uniformly spaced ripples (found in the $P_{\beta'}$ phase of pure saturated PCs).

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References

1 Yeagle, P.L. (1985) Biochim Biophys Acta 822, 267–287.
2 Rand, R.P., Parsegian, V.A., Henry, J.A.C., Lis, L.J. and McAlister, M. (1980) Can. J. Biochem. 58, 959–968.
3 Lis, L.J., McAlister, M., Fuller, N., Rand, R.P. and Parsegian, V.A. (1982) Biophys. J. 37, 657–666.
4 McIntosh, T.J., Magid, A.D. and Simon, S.A. (1989) Biochemistry 28, 17–25.
5 Ladbrooke, B.D., Williams, R.M., and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340.
6 Hui, S.W. and He, N.-B. (1983) Biochemistry 22, 1159–1164.
7 Mortensen, K., Pfeiffer, W., Sackmann, E. and Knoll, W. (1988) Biochim. Biophys. Acta 945, 221–245.
8 Copeland, B.R. and McConnell, H.M. (1980) Biochim. Biophys. Acta 599, 95–109.
9 Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1934–1954.
10 Ipsen, J.H., Karlstrom, G., Mouritsen, O.G., Wennerstrom, H. and Zuckermann, M.J. (1987) Biochim. Biophys. Acta 513, 162–172.
11 McIntosh, T.J. (1978) Biochim Biophys Acta 513, 43–58.
12 Knoll, W., Schmidt, G., Ibel, K. and Sackmann, E. (1985) Biochemistry 24, 5240–5246.
13 Inoko, Y. and Mitsui, T. (1978) J. Phys. Soc. Jap. 44, 1918–1924.
14 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733.
15 Stamatoff, J., Feuer, B., Guggenheim, H.J., Tellez, G. and Yamane, T. (1982) Biophys. J. 38, 217–226.
16 McDaniel, R.V., Simon, S.A., McIntosh, T.J. and Borovyagin, V. (1982) Biochemistry 21, 4116–4126.
17 Stewart, T.P., Hui, S.W., Portis, A.R. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 556, 1–16.
18 McIntosh, T.J., Ting-Beall, H.P. and Zampighi, G. (1982) Biochim. Biophys. Acta 685, 51–60.
19 Ruppell, D., Kapitza, H.-G., Galla, H.J., Sixl, F., and Sackmann, E. (1987) Biochim. Biophys. Acta 692, 1–17.
20 Riegler, J. and Mohwald, H. (1986) Biophys. J. 49, 1111–1118.
21 Tillack, T.W., Wong, M., Allietta, M. and Thompson, T.E. (1982) Biochim. Biophys. Acta 691, 261–273.
22 Harbich, W. and Helfrich, W. (1984) Chem. Phys. Lipids 36, 39–63.
23 Evans, E.A. and Parsegian, V.A. (1986) Proc. Natl. Acad. Sci. USA 83, 7132–7136.
24 McIntosh, T.J., Magid, A.D. and Simon, S.A. (1989) Biophys. J. 55, 897–904.
25 Nir, S. (1976) Prog. Surface Sci. 8, 1–58.
26 McIntosh, T.J. and Simon, S.A. (1986) Biochemistry 25, 4058–4066.
27 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068–6078.
28 Smith, G.S., Safinya, C.R., Roux, D. and Clark, N.A. (1987) Mol. Cryst. Liq. Cryst. 144, 235–255.
29 Smith, G.S., Sirota, E.B., Safinya, C.R. and Clark, N.A. (1988) Phys. Rev. Lett. 60, 813–816.
30 Needham, D. and Evans, E. (1988) Biochemistry 27, 8261–8269.
31 McIntosh, T.J. and Costello, M.J. (1981) Biochim. Biophys. Acta 645, 318–326.
32 Kleman, M., Williams, C.E., Costello, M.J. and Gulik-Krzywicki, T. (1977) Philos. Mag. 35, 33–56.
33 Sackmann, E. (1983) in Biophysics (Hoppe, W., Lohmann, W., Markl, H. and Ziegler, H., eds.), pp. 425–457, Springer-Verlag, Berlin and Heidelberg.
34 Israelachvili, J.N. (1985) Intermolecular and Surface Forces, pp. 150–154, Academic Press, New York.
35 LeNeveu, D.M., Rand, R.P., Parsegian, V.A. and Gingell, D. (1977) Biophys. J. 18, 209–230.
36 Goldstein, R.E. and Leibler, S. (1988) Phys. Rev. Let. 61, 2213–2216.
37 Wack, D.C. and Webb, W.W. (1989) Phys. Rev. A 40, 2712–2730.
38 Rand, R.P. and Parsegian, V.A. (1989) Biochim. Biophys. Acta 988, 351–376.
39 Marcelja, S. and Radic, N. (1976) Chem. Phys. Lett. 42, 129–130.
40 Gruen, D.W.R. and Marcelja, S. (1983) J. Chem. Soc. Faraday Trans. 2 79, 225–242.
41 Cevc, G. and Marsh, D. (1985) Biophys. J. 47, 21–32.