X-ray Diffraction Study of Three ^{19}F-Labeled Dimyristoylphosphatidylcholines

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Low-angle and wide-angle X-ray diffraction was used to examine the phase behavior, as a function of temperature, of fully hydrated multilamellar vesicles of dimyristoylphosphatidylcholine (DMPC) labeled with a difluoromethylene group in the *sn*-2 acyl chain at position 4, 8, or 12. At temperatures near 5 °C, DMPC and 2-[12,12-F_2]DMPC are both in the $L_{\beta'}$ (gel) phase with a D-spacing of 59.5 ± 0.5 Å, 2-[8,8-F_2]DMPC is in the $P_{\beta'}$ (rippled) phase with a D-spacing of 65.0 ± 0.5 Å, and 2-[4,4-F_2]DMPC is in the L_c (subgel) phase with a D-spacing of 56.5 ± 0.5 Å. Thus the greatest bilayer perturbation is caused by labeling with fluorine closest to the head group region. It was found that 2-[4,4-F_2]DMPC converted from the L_α (fluid) phase to the subgel phase at temperatures as high as 19.3 °C when incubated for 16 h at 19.3 °C. A hypothesis is presented that high concentrations of phospholipid in water (25%, w/w) facilitate the rapid formation of the subgel phase in 2-[4,4-F_2]DMPC. This hypothesis is consistent with the nucleation and growth mechanism of subgel formation as was shown for subgel formation in dipalmitoylphosphatidylcholine (DPPC).[1] When the subgel phase of 2-[4,4-F_2]DMPC was melted over the course of several days, phase coexistence of the subgel and fluid phases was observed at 23.1 °C without observation of intermediary gel or rippled phases.

Introduction

Substitution of ^{19}F for ^{1}H in the acyl chain portion of phospholipids has been used in a number of nuclear magnetic resonance (NMR) spectroscopy studies of model and biological membranes in order to take advantage of fluorine's high NMR sensitivity and low background occurrence.[2-5] Investigators have replaced a single methylene group in one or both chains with either a monofluoromethylene group or a difluoromethylene group. The thermodynamic behavior of various F-labeled phospholipids as studied by differential scanning calorimetry (DSC) has been used to monitor the perturbations to the bilayer structure caused by adding a mono- or difluoromethylene group.[6-9] For 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) labeled with a single ^{19}F at the 8- or 14-position of both chains, very little change in the main melting transition (T_m) from the unmodified lipid is observed, while a small change (–5.4 °C) is seen for the 5-F derivative.[8] A DSC study on 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine (DMPC) labeled with a difluoromethylene group at either the 8- or 12-position of both acyl chains reported a decrease in the T_m's of the 8-position derivative (–7.4 °C) and of the 12-position derivative (–3.8 °C).[7] By contrast, the 4-position derivative gave an increased T_m (+5 °C) and a broad DSC transition peak. A more recent study by Dowd et al. combined the use of ^{31}P-NMR spectroscopy and DSC to study the low-temperature behavior of DMPC labeled with a difluoromethylene group in only the *sn*-2 acyl chain at the 4-, 8-, or 12-position.[9] Both 2-[8,8-F_2]DMPC and 2-[12,12-F_2]DMPC showed decreases in their T_m's of –10.4 °C and –11 °C, respectively, but a more complex change in the melting behavior of 2-[4,4-F_2]DMPC was observed.[9]

Calorimetric measurements[9] on a 2-[4,4-F_2]DMPC sample that had been cooled to 12–16 °C from above 30 °C gave a decreased T_m (–5.5 °C) but an increased T_m (+0.9 °C) when the lipid was cooled to below 5 °C from above 30 °C. The different T_m's in 2-[4,4-F_2]DMPC were attributed to the formation of a subgel phase. Further calorimetric evidence was presented for equilibrium phase coexistence of the subgel and gel phases in the region between 5 and 12 °C, with only gel phase formed at 12 °C.[9] In the same study, however, the ^{31}P-NMR spectra showed a line shape characteristic of an anhydrous solid, or subgel phase, when 2-[4,4-F_2]DMPC was incubated for only 2.5 h at 12 °C. The authors suggested that the apparent contradiction between the calorimetric and ^{31}P-NMR results at 12 °C could be explained as a function of the experimental technique. While calorimetry measures the system as a whole, ^{31}P-NMR spectroscopy can only observe the interactions of the ^{31}P-containing head group with its surroundings.[9] In the present investigation, we use low-angle and wide-angle X-ray diffraction to elucidate the phase behavior of 2-[4,4-F]$_2$DMPC as a function of temperature. For comparison, we also perform low-angle and wide-angle X-ray diffraction of DMPC, and the DMPC derivatives labeled with a difluoromethylene group in the *sn*-2 chain at position 8 or 12.

Materials and Methods

Sample Preparation. 1,2-Dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) and 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine (DMPC) were obtained from Avanti Polar Lipids (Birmingham, AL) and used without further purification. The preparation of 1-myristoyl-2-(4,4-difluoromyristoyl)-*sn*-glycerol-3-phosphocholine (2-[4,4-F_2]DMPC), 1-myristoyl-2-(8,8-difluoromyristoyl)-*sn*-glycerol-3-phosphocholine (2-[8,8-F_2]DMPC), and 1-myristoyl-2-(12,12-difluoromyristoyl)-*sn*-glycerol-3-phosphocholine (2-[12,12-F_2]DMPC) has been described previously.[10] Thin layer chromatography (TLC) using the solvent system chloroform/methanol/7 N ammonium hydroxide (230:90:15, v/v) indicated the purity of the lipids to be at least 99%. Multilamellar vesicles (MLVs) were prepared by adding deionized, distilled water to lyophilized lipid in a 4:1 w/w ratio and cycling the dispersion three times between 70 and 5 °C with 5 min of vortexing at each temperature. Thin-walled 1.0-mm glass capillaries (Charles Supper Co.) were cleaned by sequentially washing with a chromic acid solution, deionized water, acetone, and finally copious amounts of deionized water. After being dried with nitrogen, the capillaries were flame-sealed at one end. The MLV dispersion was then loaded into the capillaries using a 1.0-mL Hamilton syringe. In order to remove air bubbles, the capillaries were centrifuged for 10 min at 1100g at 2 °C. After centrifugation, the capillaries were flame-sealed above the water layer, and this seal was dipped in silicone sealer (Dow Corning Corp., Midland, MI). Upon completion of the diffraction experiments,

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it was confirmed that the samples had remained fully hydrated by observing the presence of the excess water layer above the opaque lipid dispersion. TLC was performed on all the samples after X-ray diffraction to check for degradation.

X-Ray Diffraction. The X-ray source was an Elliott rotating anode Type GX21, typically operated at 4.2 kW. After each X-ray exposure, the sample was vertically translated by 1–2 mm relative to the beam. This ensured that X-ray data were obtained from the sample that had not been previously exposed to radiation, since it was found in a previous study that subgel formation in DPPC is inhibited by radiation damage.[1]

Film Data. The X-ray beam was passed through a nickel filter to select Cu Kα radiation and pinhole collimated (two 0.3-mm holes 6.5 cm apart), and the patterns recorded on Kodak DEF5 X-ray film (Charles Supper Co., Natick, MA) at a distance of 60 mm from the sample. The temperature was controlled to ±0.5 °C using a YSI Model 72 Proportional Temperature Controller (Yellow Springs, OH) connected to a quartz heater. This heater and a finned coil cooled by a Model KR60A chiller (Polysciences Corp., Niles, IL) were located within the insulated 2 × 2 × 2 ft radiation shield surrounding the sample and camera. Six small fans circulated air inside the chamber. One insulated TFD probe (Omega Engineering, Inc., Stamford, CT) which had been calibrated to a NIST thermometer (Taylor Instrument Co., Rochester, NY) monitored the temperature in the aluminum sample holder equipped with 1.5 μm thick Mylar windows (Dupont, Wilmington, DE). The sample was mounted 0.5 cm from the insulated TFD probe and cooled through the air. The temperature gradient between the insulated TFD probe and the sample was measured in a separate experiment as a function of temperature using a precalibrated, uninsulated TFD probe (Omega) glued to the capillary. The temperature was about 0.5 °C higher at the sample than in the aluminum sample holder; temperatures quoted are at the sample. Each X-ray exposure took 2–3 h unless stated otherwise.

Microscopy. The microscopy of DPPC was carried out using a Nikon polarized-light microscope at room temperature. Hydrated samples were placed onto a microscope slide and covered with a coverslip.

Results and Discussion

The first objective of this study was to verify the suggestion that the 2-[4,4-F_2]DMPC derivative forms a subgel phase when incubated below 5 °C and to obtain the X-ray diffraction signature of a well-formed subgel phase in this derivative. To this end, the 2-[4,4-F_2]DMPC sample was first heated above 40 °C to melt any preexisting subgel or gel phases and then incubated in the radiation chamber at 3.2 °C for 16 h. The resulting X-ray diffraction film data are shown in Figure 1A. In the wide-angle region, the picture is very different from that of DMPC gel phase data (see Figure 2A). Instead of only two wide-angle reflections resulting from the orthorhombic chain packing in the gel phase, many reflections are observed in the wide-angle region of Figure 1A due to the highly ordered packing of phospholipids in the subgel phase. The numerous reflections are similar to those observed for the subgel phase in DPPC[1,11-13] and are summarized in Table 1. This result, that a well-formed subgel is obtained after a 16-h 3.2 °C incubation, is consistent with the low-temperature calorimetric results obtained by Dowd et al.[9] In that work, 2-[4,4-F_2]DMPC that had been cooled to below 5 °C and then scanned by differential scanning calorimetry (DSC) at 15 °C/h showed only a subtransition with no main transition (gel-to-fluid phase), indicating that the sample had been fully converted to the subgel phase in roughly 1 1/2 h below 5 °C.

In the same study,[9] a 2-week incubation of 2-[4,4-F_2]DMPC at 12 °C produced a ^{31}P-NMR spectrum typical of an anhydrous solid, consistent with a subgel phase, and different from a gel phase signal. In order to determine the time course of formation

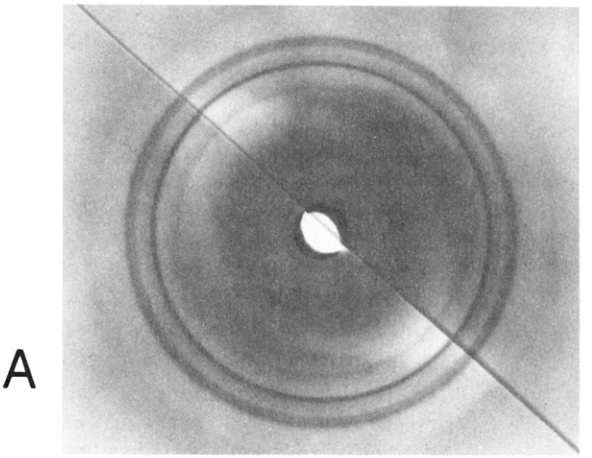

Figure 1. Subgel phase: (A) 2-[4,4-F_2]DMPC was incubated for 16 h at 3.2 °C and then X-rayed at 4.8 °C; (B) 2-[4,4-F_2]DMPC was incubated for 3 h at 12.8 °C and then X-rayed at 12.8 °C. The reflections are slightly offset due to a slightly smaller sample-to-film distance in part A.

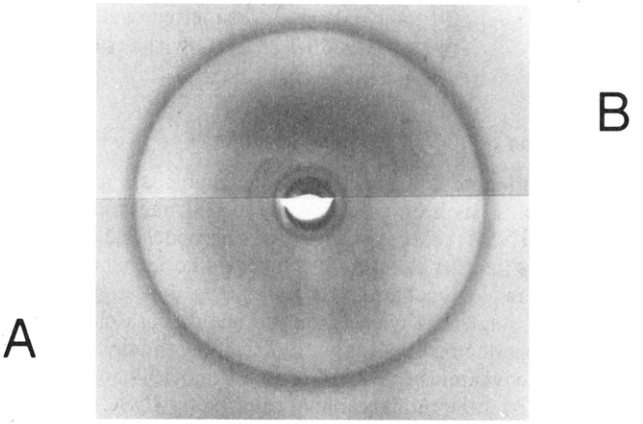

Figure 2. Gel phase: (A) DMPC was incubated for 4 days at 4–7 °C and then X-rayed at 5.5 °C; (B) 2-[12,12-F_2]DMPC was incubated for 4 h at 3.1 °C and then X-rayed at 4.6 °C.

TABLE 1: X-ray D-Spacings of the Subgel Phase[a]

DPPC (Å)	2-[4,4-F_2]DMPC (Å)
Low Angle	
59.4 ± 0.1	56.5 ± 0.5
Wide Angle	
10.14 (medium)	
9.36 (weak)	9.4 (medium)
8.6 (very weak)	8.8 (weak)
	7.8 (very weak)
6.84 (medium)	7.0 (very weak)
	5.10 (medium)
4.46 (strong)	4.4 (strong)
4.25 (very weak)	4.2 (very weak)
3.92 (broad)	4.0 & 3.9 (medium doublet)
	3.3 (very weak)

[a] The 2-[4,4-F_2]DMPC D-spacings were obtained using film data. The DPPC subgel D-spacings were obtained from data in ref 1.

of the subgel-like spectrum, Dowd et al. followed the ^{31}P-NMR signal as a function of time at 12 °C.[9] Figure 8 in ref 9 shows that the anhydrous solid-type spectrum appeared after only 2 h. When we incubated 2-[4,4-F_2]DMPC at 12.8 °C in the X-ray capillary, even a short 3-h incubation produced the subgel phase as shown by the X-ray film in Figure 1B. The pattern is identical to that produced by incubating 2-[4,4-F_2]DMPC for 16 h at 3.2 °C (Figure 1A), yielding the same D-spacings as shown in Table 1. This dramatic result is consistent with the ^{31}P-NMR time course study that showed the appearance of a subgel-like spectrum

in only 2 h at 12 °C. This result is *not* consistent, however, with the calorimetry data that showed that only the gel phase was formed at 12 °C for varying times of incubation.[9]

The question then is why did the 2-[4,4-F_2]DMPC *not* form subgel when held in the calorimeter at 12 °C for various incubation times and why *did* it form subgel when held in the X-ray capillary for 3 h at 12 °C or in the NMR tube for only 2 h at 12 °C? One explanation may be the effect of lipid concentration. For calorimetry, very dilute samples are used, typically a 1000:1 water-to-lipid weight ratio, while for ^{31}P-NMR the weight ratio is 5–8:1 and for X-ray diffraction it is 3–4:1. For a different phospholipid, Silvius et al.[14] have shown that the rate of subgel formation in 1,2-diisopalmitoyl-*sn*-glycerol-3-phosphoethanolamine (DIPPE) as monitored by DSC is much faster when a 4:1 water-to-lipid weight ratio is used than when a 200:1 weight ratio is used. A similar explanation has been suggested for the well-studied phospholipid DPPC.[1] It has been shown using DSC that when DPPC is incubated at 0.1 °C, a metastable subgel intermediate decays slowly into the more stable subgel form with a half-life of 18 days.[15] However, the conversion of a metastable subgel intermediate in DPPC formed at 2.3 °C to the more stable subgel form occurs in only 3 days when observed by X-ray diffraction.[1] Thus the differences observed by Dowd and colleagues in the calorimetric and ^{31}P-NMR results[9] may have been due to differences in concentration.

Since the mechanism of subgel formation in DPPC is basically one of nucleation of the new subgel phase in the gel phase, followed by growth of the subgel domains,[1,15–17] it is possible that growth of subgel domains is facilitated by higher concentrations of lipid. When a 1000:1 water-to-lipid weight ratio preparation of DPPC was examined using polarized light microscopy, the circular morphology of the MLVs produced a maltese-cross appearance first described for MLVs in ref 18. The MLVs are approximately 1–5 μm in diameter and remain isolated from each other. In a 3:1 preparation, however, a majority of the MLVs are again 1–5 μm in diameter but are now closely packed with their edges in contact. It is possible that subgel phase nucleated and grown in one MLV can then grow into an adjacent MLV, and so on. Thus the effective growth domain would be much larger in the case of closely packed MLVs, rather than terminating at the edge of a single MLV as in the case for a dilute solution. This would enhance the rate of conversion of the metastable intermediate subgel to the more stable form of the subgel.

Tenchov[19] has described the reversibility of many lipid phase transitions in water, paying particular attention to the metastability of phases during heating and cooling. The lipid 2-[4,4-F_2]DMPC falls into category A in Figure 2 of ref 19, which is defined as a type of lipid which displays metastability in both the fluid (L_α) phase and the gel ($L_{\beta'}$) phase with respect to the subgel phase below the subtransition temperature. In these lipids the subtransition occurs at a higher temperature than the main gel-to-fluid transition. Similar behavior was shown for short-chain phosphatidylethanolamines, 1,2-dilauroyl-[20,21] and 1,2-dimyristoyl-*sn*-glycerol-3-phosphoethanolamine.[22]

For comparison, no subgel formation occurred when DMPC was incubated for 4 days at temperatures between 4 and 7 °C (Figure 2A). This is not surprising since Lewis et al.[23] have shown that quite aggressive measures at very low temperatures are required to nucleate and grow the subgel phase in DMPC. The D-spacing of the lamellar repeat of the DMPC gel phase at 6.5 °C is 59.6 ± 0.4 Å. Similarly, when 2-[12,12-F_2]DMPC was incubated for 4 h at 3.1 °C, no subgel phase was observed, but instead a typical gel phase X-ray pattern was obtained as shown in Figure 2B. The D-spacing of the lamellar repeat of the film in Figure 2 is 59.3 ± 0.5 Å, in agreement with that of DMPC. No change in D-spacing was seen when the sample of 2-[12,-12-F_2]DMPC in Figure 2B was incubated for an additional 20 h at 5 °C. Thus, replacing a CH_2 group with a CF_2 group near the center of the bilayer does not enhance the rate of subgel formation in these lipids. McDonough et al.[8] also noted that substituting a monofluoromethylene group into both chains near the center of the DMPC bilayer produced the smallest perturbation in T_m compared to F-labeling at other positions.

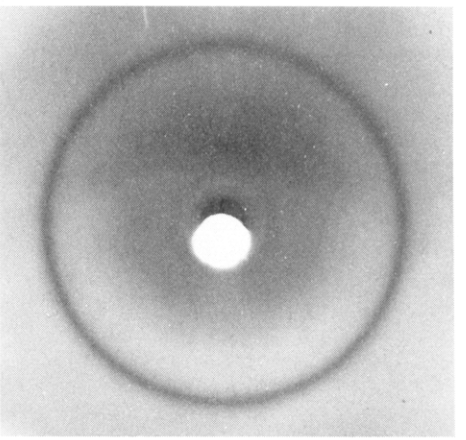

Figure 3. Rippled phase: 2-[8,8-F_2]DMPC was incubated for 2 days between 2.6 and 6.6 °C and then X-rayed at 5.5 °C for 8 h.

When 2-[8,8-F_2]DMPC was incubated for 2 days between 2.6 and 6.6 °C and then X-rayed at 5.5 °C, the reflections were typical of the rippled ($P_{\beta'}$) phase. The film data are shown in Figure 3; they yield a lamellar repeat of 65.0 ± 0.5 Å, which is larger than that of the DMPC gel phase (59.6 ± 0.4 Å) and the same as that of the DMPC rippled phase.[24] As shown in Figure 3, there is a single wide-angle reflection at 4.2 Å, resulting from the nearly hexagonal packing in the rippled phase.[25] Thus, replacing a CH_2 group with a CF_2 group near the center of the chain either stabilizes the rippled phase or destabilizes the gel phase relative to DMPC at 5.5 °C.

Slow Melting of the Subgel in 2-[4,4-F_2]DMPC. Although an investigation of the exact temperature requirements for subgel formation in 2-[4,4-F_2]DMPC was not carried out, we found that the subgel phase could be formed in this derivative in the X-ray capillary by incubating at temperatures even as high as 19.3 °C. In one experiment a fresh sample of 2-[4,4-F_2]DMPC was first heated to above 50 °C, allowed to equilibrate at 19.3 °C overnight, and then X-rayed at 20 °C. The well-defined subgel phase formed by these procedures (film not shown) was subsequently slowly melted over the course of several days in order to clarify the calorimetric results of Dowd et al.[9] In that work, a DSC scan showed a broad subtransition in 2-[4,4-F_2]-DMPC which was fitted to two independent, two-state transitions.[9] Dowd et al. noted that the data were consistent with the existence of two solid phases but could also be caused by a single phase undergoing a transition consisting of two independent or sequential two-state steps. To investigate these possibilities, the temperature of the X-ray capillary was first raised from 20 to 21.1 °C and held there for 22 h. X-ray diffraction showed the sample to be still in the subgel phase. The next step in temperature was to 23.1 °C for 48 h. At this temperature, evidence for a new phase coexisting with the subgel was seen in the low-angle data, although only one new reflection was observed (Figure 4A). The new reflection appears between the $h = 1$ and $h = 2$ low-angle reflections of the residual subgel phase. This single new reflection is only consistent with the appearance of the fluid phase, as will be shown, and is not consistent with a second solid phase. The temperature of 23.1 °C agrees with the DSC result showing the onset of the subtransition at this temperature, although in the earlier study[9] the subtransition temperature may have been slightly elevated due to the non-zero scan rate of 15 °C/h.

The next step in temperature was to 24.2 °C for 19 h. At this temperature the subgel was completely absent and was replaced by the fluid phase, as shown in Figure 4B. In the fluid phase only

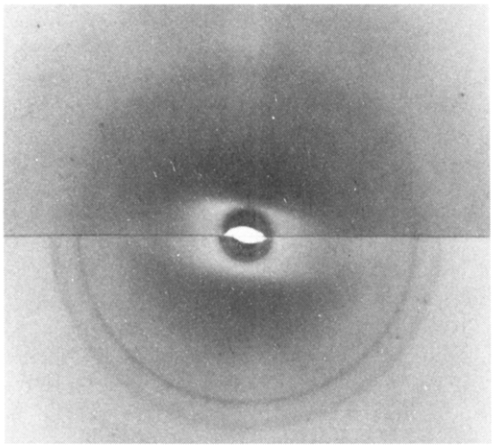

Figure 4. (A) Phase coexistence of subgel and fluid phases. 2-[4,4-F_2]-DMPC in the subgel phase was heated to 23.1 °C and held there for 48 h before being X-rayed at 23.1 °C. (B) Fluid phase. The sample in 4A was heated to 24.2 °C and held there for 19 h before being X-rayed at 24.2 °C. The amorphous dark shadows in these films are due to X-ray scattering off the edge of the beam stop.

the $h = 1$ and $h = 2$ low-angle lamellar reflections are visible with a D-spacing of 65 ± 5 Å and one broad, light wide-angle reflection with a D-spacing of 4.6 Å. (A large error in the low-angle D-spacing in the fluid phase results from the small number of sharp reflections for measurement.) The half-width of the wide-angle reflection is somewhat smaller than that observed for other fluid phase phosphatidylcholines,[26,27] suggesting less freedom of chain movement in this derivative in the fluid phase than in DMPC. It is perhaps that gauche rotamers can only form below the 4-position CF_2 group, since this group may impart a nearly rigid structure to the upper third of the 2-acyl chain. This could lead to greater ease of chain packing during the fluid-to-subgel phase transformation. The pattern seen in Figure 4B persisted after the temperature was increased to 30.6 °C, where the sample was held for 16 h. The final step in the melting was to increase the temperature to 51.2 °C and hold the sample there for 19 h. At this temperature the overall pattern is similar (film not shown) to that in Figure 4B with two low-angle reflections and one wide-angle reflection with the same half-width as shown in Figure 4B. After this slow-melting experiment, TLC revealed the degradation in this sample to be 5–10%. This amount of lysolecithin is larger than the <1% degradation of all the other irradiated lipids due to the higher temperatures and longer exposure times in this experiment. However, the results described here are not invalidated by this 5–10% degradation, since the capillary was moved vertically 1–2 mm to a new position in the beam for each picture.

Thus, the X-ray diffraction results obtained during the slow melting of the subgel phase in 2-[4,4-F_2]DMPC do not support the hypothesis that two solid phases melt during the subtransition, since neither a gel nor a rippled phase was observed. Rather, phase coexistence of the subgel and the fluid phase occurred at 23.1 ± 0.5 °C with several subgel wide-angle reflections simultaneously present with only one low-angle fluid phase reflection.

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References and Notes

(1) Tristram-Nagle, S.; Suter, R. M.; Sun, W.-J.; Nagle, J. F. *Biochim. Biophys. Acta* **1994**, *1191*, 14–20.
(2) Ho, C.; Dowd, S. R.; Post, J. F. M. *Curr. Top. Bioenerg.* **1985**, *14*, 53–95 and references therein.
(3) MacDonald, P. M.; Sykes, B. D.; McElhaney, R. N. *Biochemistry* **1985**, *24*, 2412–2419.
(4) Peng, Z.-Y.; Tjandra, N.; Simplaceanu, V.; Ho, C. *Biophys. J.* **1989**, *56*, 877–885.
(5) McElhaney, R. N. *CRC Crit. Rev. Microbiol.* **1989**, *17*, 1–32 and references therein.
(6) Longmuir, K. J.; Capaldi, R. A.; Dahlquist, F. W. *Biochemistry* **1977**, *16*, 5746–5755.
(7) Sturtevant, J. M.; Ho, C.; Reimann, A. *Proc. Nat. Acad. Sci. U.S.A.* **1979**, *76*, 2239–2243.
(8) MacDonald, P. M.; McDonough, B.; Sykes, B. D.; McElhaney, R. N. *Biochemistry* **1983**, *22*, 5097–5103.
(9) Dowd, S. R.; Sturtevant, J. M.; Simplaceanu, V.; Ho, C. *J. Phys. Chem.* **1993**, *97*, 2946–2951.
(10) Engelsberg, M.; Dowd, S. R.; Simplaceanu, V.; Ho, C. *Biochemistry* **1982**, *21*, 6985–6989.
(11) Ruocco, M. J.; Shipley, G. G. *Biochem. Biophys. Acta* **1982**, *684*, 59–66.
(12) Füldner, H. H. *Biochemistry* **1981**, *20*, 5707–5710.
(13) Church, S. E.; Griffiths, D. J.; Lewis, R. N. A. H.; McElhaney, R. N.; Wickman, H. H. *Biophys. J.* **1986**, *49*, 597–605.
(14) Silvius, J. R.; Lyons, M.; Yeagle, P. L.; O'Leary, T. J. *Biochemistry* **1985**, *24*, 5388–5395.
(15) Tristram-Nagle, S.; Wiener, M. C.; Yang, C.-P.; Nagle, J. F. *Biochemistry* **1987**, *26*, 4288–4294.
(16) Yang, C. P.; Nagle, J. F. *Phys. Rev. A* **1988**, *37*, 3993–4000.
(17) Pali, T.; Bartucci, R.; Horvath, L. I.; Marsh, D. *Biophys. J.* **1993**, *64*, 1781–1788.
(18) Tristram-Nagle, S.; Wingert, L. M. *Mol. Cryst. Liq. Cryst.* **1990**, *188*, 41–56.
(19) Tenchov, B. *Chem. Phys. Lipids* **1991**, *57*, 165–177.
(20) Chang, H.; Epand, R. M. *Biochim. Biophys. Acta* **1983**, *728*, 319–324.
(21) Seddon, J. M.; Harlos, K.; Marsh, D. *J. Biol. Chem.* **1983**, *258*, 3850–3854.
(22) Wilkinson, D. A.; Nagle, J. F. *Biochemistry* **1984**, *23*, 1538–1541.
(23) Lewis, R. N. A. H.; Mak, N.; McElhaney, R. N. *Biochemistry* **1987**, *26*, 6118–6126.
(24) Janiak, M. J.; Small, D. M.; Shipley, G. G. *Biochemistry* **1976**, *15*, 4575–4580.
(25) Hentschel, M. P.; Rustichelli, F. *Phys. Rev. Lett.* **1991**, *66*, 903–906.
(26) Ranck, J. L.; Mateu, L.; Sadler, D. M.; Tardieu, A.; Gulik-Krzywicki, T.; Luzzati, V. *J. Mol. Biol.* **1974**, *85*, 249–277.
(27) Rand, R. P.; Chapman, D.; Larsson, K. *Biophys. J.* **1975**, *15*, 1117–1124.