Magnetic Nonequivalence of the Two Fatty Acid Chains in Phospholipids of Small Unilamellar Vesicles and Mixed Micelles†

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ABSTRACT: Magnetic nonequivalence of the two acyl chains in phospholipids has been shown by ^{1}H NMR in several kinds of micelles [Roberts, M. F., Bothner-By, A. A., & Dennis, E. A. (1978) *Biochemistry*, *17*, 935]. The chemical shifts of the two α-methylene groups have been assigned, and a difference of about 0.1 ppm between the two chains has been interpreted as a difference in the position of the two fatty acid chains relative to the interface. This conclusion has now been extended to phospholipid systems closer to biological membranes in the form of small unilamellar vesicles prepared by sonication of phospholipid dispersions. Several kinds of phospholipid vesicles are compared, including phosphatidylserine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidic acid, and phosphatidylcholine. By using suitable resolution enhancement techniques, it was possible to overcome the broad lines in the spectra and observe the *sn*-1 and *sn*-2 α-methylene protons in most of the phospholipid vesicles. The two α-methylene protons on the *sn*-2 chain are nonequivalent, and the parameters of the AB pattern can be deduced ($\Delta\nu_{AB}$ = 0.02 ppm, J_{AB} = 17 Hz). In contrast, the α-methylene protons of the *sn*-1 chain are not magnetically distinguishable. The same pattern is also found when the resolution enhancement techniques are used with mixed micelles of Triton X-100 and long-chain phospholipids. This result confirms the idea of one main conformation or three-dimensional structure for phospholipids at the lipid–water interface in all aggregated states investigated (crystal, bilayer, and micelle). In this conformation, the first methylene group on the *sn*-2 chain is positioned at the interface, and the two protons appear nonequivalent on the NMR time scale. On the other hand, the α-methylene protons of the *sn*-1 chain are localized in a more hydrophobic environment and appear magnetically equivalent on the NMR time scale. The pattern obtained with vesicles made from zwitterionic phospholipids such as phosphatidylcholine is more complicated to interpret than those of anionic phospholipids, probably due to tighter packing at the interface.

Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) has been used to show that the two phospholipid acyl chains are nonequivalent in micellar systems (Roberts & Dennis, 1977; Roberts et al., 1978). In all cases investigated, a difference of about 0.1 ppm between the two α-methylene groups has been found. Moreover in the case of the best resolved spectra (mixed micelles of short-chain lipids with Triton[1] and mixed micelles of long-chain lipids with ionic detergents), the fine structure of the signal was resolvable, showing the further magnetic nonequivalence of the two α-methylene protons on the *sn*-2 chain. This nonequivalence has also been indicated in other phospholipid aggregates by various physical techniques. The crystalline structure of dilauryl-PE[1] (Hitchcock et al., 1974) and dimyristoyl-PC (Pearson & Pasher, 1979) has been investigated. In both structures, the *sn*-2 chain starts parallel to the bilayer surface while the *sn*-1 chain starts perpendicular. Consequently, the α-methylene carbons of the two chains are out of step by about 3–4 carbon atoms with respect to the interface. Neutron diffraction studies of specifically deuterated phosphatidylcholine in the Lβ phase led to the calculation of a difference of 1.8 Å (1.5 carbon–carbon bond lengths) in the position of corresponding segments with respect to the interface (Buldt et al., 1978; Zaccai et al., 1979). The nonequivalence in the packing of the two chains in multibilayers has also been observed by intermolecular cross-linking experiments (Gupta et al., 1979) and Raman spectroscopy (Gaber & Petitcolas, 1978).

Several studies have been carried out with deuterium NMR on phospholipids with various polar head groups in multibilayer preparations (Seelig & Seelig, 1974, 1975; Seelig & Browning, 1978; Gally et al., 1979; Browning & Seelig, 1980; Seelig et al., 1980) and on multibilayers of saturated PC in the presence of cholesterol (Haberkorn et al., 1977; Oldfield et al., 1978). In all cases the quadrupole splittings show the magnetic nonequivalence of the two chains at the α-methylene position and are interpreted according to the X-ray and neutron diffraction structures.

Small unilamellar vesicles are one of the most widely used membrane models even though their single bilayer has a higher curvature than natural biological membranes (Sheetz & Chan, 1972; Seiter & Chan, 1973), and it is important to characterize the phospholipid structure of such systems. Unfortunately, deuterium NMR cannot be used because the quadrupole splittings disappear due to the fast isotropic tumbling of the vesicles (Stockton et al., 1974, 1976). ^{1}H NMR of small unilamellar vesicles gives a much broader spectrum than micelles, and fine structure in the α-methylene region has not been previously reported. Resolution enhancement methods have been used successfully to resolve broad and overlapping lines in spectra of proteins and peptides (Campbell et al., 1973; De Marco & Wüthrich, 1976). We have now employed similar methods (Clin et al., 1979) with several kinds of phospholipid vesicles and mixed micelles of long-chain phospholipids with nonionic surfactants and can now extend our previous ^{1}H NMR studies to these important systems.

Experimental Procedures

Materials. PC was prepared from egg yolk according to Singleton et al. (1965). PE prepared by transesterification of egg PC was obtained from Avanti Biochemicals. Egg PA

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[1] Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid; Triton, Triton X-100; TSP, sodium 3-(trimethylsilyl)propionate-*2,2,3,3-d*$_4$; cmc, critical micelle concentration.

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Table I: Spectral Data for α-Methylene Protons of Phospholipids in Mixed Micelles and Vesicles

phospholipid	pD	*sn*-1 ν (ppm)	*sn*-2 ν (ppm)	*sn*-2 $\Delta\nu_{AB}$ (ppm)	*sn*-2 J_{AB} (Hz)	$\Delta\nu_{sn\text{-}2\text{-}sn\text{-}1}$ (ppm)	$J_{\alpha\beta}$ (Hz) *sn*-1	$J_{\alpha\beta}$ (Hz) *sn*-2
Triton/PA	7.2	2.21	2.32	0.018	17	0.11		
Triton/PS	8.7	2.28	2.40	0.018	16	0.12		
Triton/PG	6.6	2.24	2.35	0.018	15	0.11		
Triton/PC	6.5	2.24	2.37	0.018	15	0.13		
Triton/PE	6.3	2.23	2.34	0.019	18	0.11		
PA vesicles	7.9	2.35	2.42	0.018	18	0.07	7	6
PS vesicles	7.4	2.34	2.42	0.017	16	0.08	8	6
PG vesicles	7.4	2.35	2.41	0.018	17	0.06	7	7
PE vesicles	9.1	2.35	2.41	0.018	18	0.06	7	7

and PG were obtained from Calbiochem. PS was purified from bovine brain according to Folch (1942) and acid washed according to Rathbone et al. (1962) in order to eliminate divalent cations. All of the phospholipids gave a single spot on thin-layer chromatography with the solvent system $CHCl_3/CH_3OH/H_2O$ (65:25:4 v/v/v) and were stored at −10 °C in benzene annd lyophilized prior to use. Triton X-100 was obtained from Rohm and Haas.

Vesicle Preparation. A 50-mg sample of pure dry phospholipid was dispersed in 2 mL of D_2O, and the pD was adjusted by the addition of NaOD. The dispersion was mixed by vortexing and was then sonicated at 4 °C under nitrogen with an MSE sonicator until the solution turned clear (3–10 min). The pD was checked again, and if adjusted, the solution was resonicated. The sonicated dispersions were calibrated by elution through a cross-linked Sepharose 2B column. PC, PS, PA, and PG eluted at a volume corresponding to small unilamellar vesicles. Sonicated preparations of PE at pD 9.0 aggregated on the column so they could not be calibrated.

Mixed Micelle Preparation. Dry phospholipids were dispersed in 0.5 mL of D_2O, and a solution of Triton X-100 in D_2O was added to give final concentrations of 10 mM phospholipid and 40 mM Triton. The solution was vortexed to ensure total mixing, and the pD was adjusted when necessary.

^1H NMR. ^1H NMR experiments were performed either at Stanford University or at the University of California, San Diego, with a Bruker 360-MHz NMR spectrometer equipped with quadrature phase detection and either a Nicolet 1080FT or Nicolet 1180FT system. Experimental conditions were the following: sweep width = ±1500 for the vesicles and ±1800 for the mixed micelles. An 8K memory was employed to accumulate 200 scans for the nondecoupled and 500 scans for the decoupled spectra. For the regular spectrum, a line broadening of 0.5 Hz was applied in order to reduce the noise. Chemical shifts were measured with an accuracy of ±0.1 Hz. Enhanced spectra were obtained by the exponential sine multiplication method of Clin et al. (1979). The natural line width of the α-methylene protons is about 15 Hz. Lines were generally broadened by 1 Hz, and a 2-Hz separation was used between the two dispersion spectra. In the enhanced spectra which were used for the determination of $\Delta\nu_{AB}$, J_{AB}, and $J_{\alpha\beta}$, the chemical shifts were measured with an accuracy of ±1 Hz (0.003 ppm). TSP was included in all samples as a chemical shift standard. All spectra were obtained at 22 °C.

Results

Mixed Micelles of Triton X-100 and Long-Chain Phospholipids. The ^1H NMR spectrum of Triton/PA mixed micelles is shown in Figure 1. The main peaks of Triton and phospholipids have been previously assigned (Ribeiro & Dennis, 1975; Roberts et al., 1978; Dennis et al., 1979). The insert corresponds from right to left to peaks for the CH_2—C=C (2.0 ppm), CH_2—C=O (2.2–2.4 ppm), and C=C—CH_2—C=C (2.8 ppm) protons. The two peaks at 2.21 and 2.32 ppm can be assigned to the *sn*-1 and *sn*-2 α-methylene groups, respectively, in analogy to the assignment in Triton/PC micelles (Roberts & Dennis, 1977). In that case, the assignment was based on a comparison with the spectrum of PC in which the α-methylene group of the *sn*-2 fatty acid was deuterated. In insert A of Figure 1, these two peaks are well separated, but no fine structure is observed. In the enhanced spectrum (insert B of Figure 1), the two peaks are better resolved, but fine structure is still not clear. In the β-methylene decoupled spectrum (insert C of Figure 1), a singlet is apparent for the *sn*-1 chain, and a quartet can be resolved for the *sn*-2 chain, as is observed for short-chain PC in mixed micelles with Triton (Roberts et al., 1978). They correspond to an A_2 system for the *sn*-1 chain and an AB quartet of $\Delta\nu_{AB} = 0.018 \pm 0.003$ ppm and $J_{AB} = 17.0 \pm 1$ Hz for the *sn*-2 chain. This spectrum clearly shows (i) the nonequivalence of the α-methylene protons on the two chains and (ii) the further nonequivalence of the two α-methylene protons on the *sn*-2 chain. This is in agreement with previous results on short-chain PC in mixed micelles with Triton and long-chain PC in mixed micelles with ionic detergents.

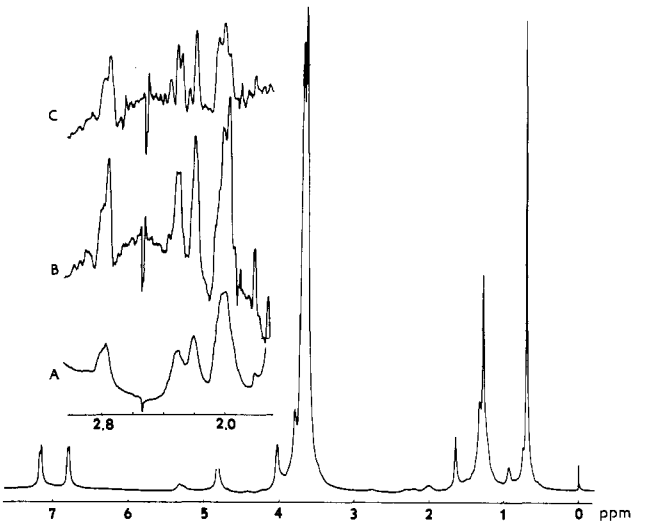

FIGURE 1: ^1H NMR spectrum (360 MHz) of Triton/PA mixed micelles at a molar ratio of 4:1 Triton phospholipid and pD 7.2. Insert A is the expanded part of the spectrum between 2.0 and 2.8 ppm which shows the α-methylene peaks (2.2–2.3 ppm). Insert B is the same as insert A with resolution enhancement. Insert C is the expanded part of the spectrum with the β-methylene protons decoupled and resolution enhancement.

Figure 2 shows the α-methylene spectrum of mixed micelles of Triton and four different phospholipids, PS, PG, PC, and PE. In each case, one can detect in the β-methylene decoupled spectrum a singlet for the *sn*-1 α-methylene protons and an AB quartet for the *sn*-2 α-methylene protons with $\Delta\nu_{AB} = 0.018 \pm 0.003$ ppm and $J_{AB} = 15$–18 Hz. Specific values for

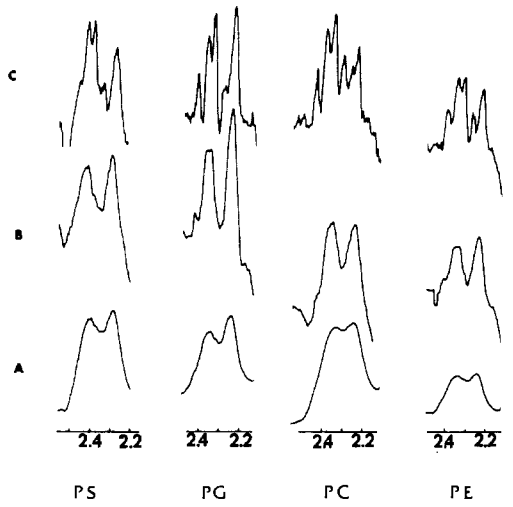

FIGURE 2: ^{1}H NMR spectrum in the 2.2–2.4-ppm region of Triton/PS, Triton/PG, Triton/PC, and Triton/PE mixed micelles at a molar ratio of 4:1 Triton phospholipid and pD 8.7, 6.6, 6.5, and 6.3, respectively, Spectra A are without and spectra B are with resolution enhancement. Spectra C are with the β-methylene protons decoupled and resolution enhancement.

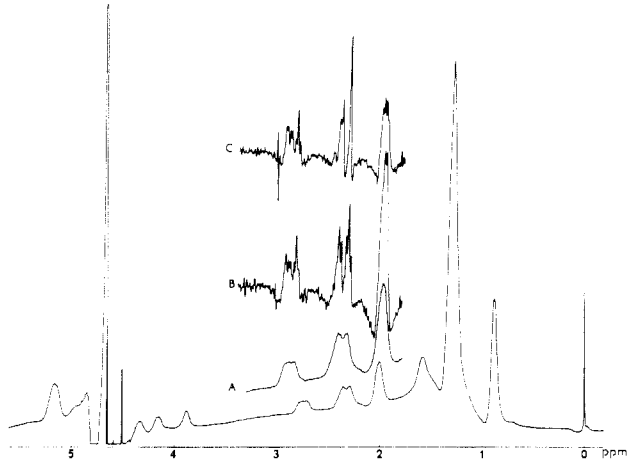

FIGURE 3: ^{1}H NMR spectrum of small unilamellar vesicles of PA at a concentration of 25 mM and pD 7.9. Insert A is the expanded part of the spectrum between 2.0 and 2.8 ppm which shows the α-methylene peaks (2.35–2.42 ppm). Insert B is the same as insert A with resolution enhancement. Insert C is the expanded part of the spectrum with the β-methylene protons decoupled and resolution enhancement.

each kind of micelle are reported in Table I.

Phospholipid Vesicles. The ^{1}H NMR spectrum of PA in small unilamellar vesicles in shown in Figure 3. By analogy to the assignment in Figure 1, the peaks at 2.35 and 2.42 ppm are clearly distinguishable and correspond to the α-methylene protons, although they are somewhat downfield (by 0.1 ppm) by comparison with the mixed micelles of Triton and PA. Insert B of Figure 3 shows a triplet at 2.35 ppm with a coupling constant $J_{\alpha\beta}$ of 7.3 Hz and a multiplet at 2.42 ppm which is not clearly resolved. In the β-decoupled spectrum (Figure 3, insert C), the triplet simplifies to a singlet, while the pattern at 2.42 ppm shows an AB quartet of $\Delta\nu_{AB} = 0.018 \pm 0.003$ ppm and $J_{AB} = 18 \pm 1$ Hz.

Figure 4 shows the α-methylene spectrum of PS, PG, and PE vesicles. As in the two previous spectra, the peaks at 2.35 and 2.42 ppm are clearly distinguishable without resolution enhancement. In insert C of Figure 4 (β-decoupled spectrum), the two peaks can be assigned as a singlet at 2.35 ppm and an AB quartet of $\Delta\nu_{AB} = 0.018 \pm 0.003$ ppm and $J_{AB} = 17 \pm 1$ Hz, as previously observed for PA vesicles. In insert B

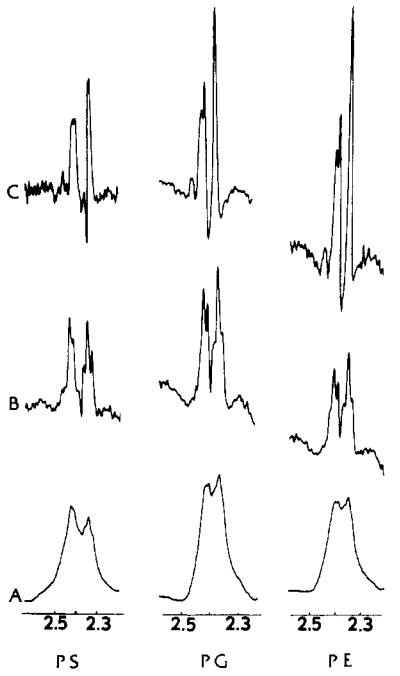

FIGURE 4: ^{1}H NMR spectrum of the 2.3–2.5-ppm region of sonicated vesicles of PS, PG, and PE at a concentration of 25 mM and pD 7.4, 7.4, and 9.1, respectively, Spectra A are without and spectra B are with resolution enhancement. Spectra C are with the β-methylene protons decoupled and resolution enhancement.

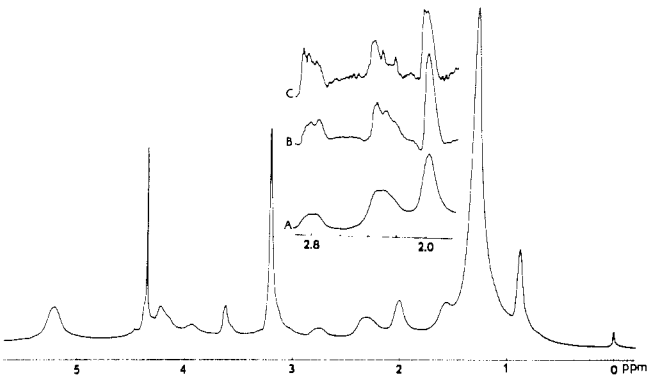

FIGURE 5: ^{1}H NMR spectrum of small unilamellar vesicles of PC at a concentration of 25 mM and pD 6.9. Insert A is the expanded part of the spectrum between 2.0 and 2.8 ppm which shows the α-methylene protons. Insert B is the same as insert A with resolution enhancement. Insert C is the expanded part of the spectrum with the β-methylene protons decoupled and resolution enhancement.

of Figure 4, there is a triplet of $J_{\alpha\beta} = 7 \pm 1$ Hz for the *sn*-1 chain. Moreover the separation between the two central lines of the *sn*-2 α-methylene multiplet is about the same, 6.5 Hz, a value which corresponds to a ^3J coupling with protons in free rotation. Specific values for each kind of vesicle are reported in Table I.

The spectrum of small unilamellar vesicles of egg PC is shown in Figure 5. In contrast with the previous spectra, only one main peak is observed around 2.35 ppm, although it is not symmetrical. The enhanced spectrum shows a complicated pattern, and the β-methylene decoupled and enhanced spectrum is not fully resolvable, although three main resonances at 2.26, 2.34, and 2.39 ppm can be observed.

Discussion

Mixed micelles of PC, PE, and PS in Triton X-100 have been studied by ^{1}H NMR at 220 MHz (Roberts & Dennis, 1977). In all cases, the α-methylene signal gave two broad overlapping peaks with a difference of 0.1 ppm. By using

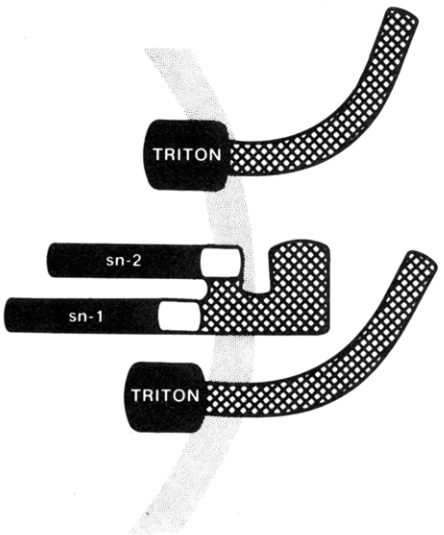

FIGURE 6: Schematic picture of a phospholipid molecule at the lipid–water interface in a mixed micelle with Triton X-100. The black and hatched areas correspond to hydrophobic and hydrophilic portions of the molecules, respectively, and the white areas correspond to the regions containing the α-methylene groups on the *sn*-1 and *sn*-2 fatty acyl chains.

enhancement methods and higher field, we are now able to obtain the fine structure of those two signals. The *sn*-1 α-methylene protons appear as magnetically equivalent due to a fast and isotropic reorientation of the CH_2 segments. On the other hand, the *sn*-2 α-methylene protons give an AB spectrum probably because they are more restricted at the interface. We found a similar pattern for PA, PG, PE, PC, and PS mixed micelles with Triton. These results together with the earlier results on short-chain lipid micelles and long-chain lipid micelles of PC with ionic detergents show that the lipid conformation at a mixed micelle interface does not depend principally on the chain length or on the polar head group of the phospholipid.

The conformation of phospholipids in various aggregation states is now well-defined with the aid of a number of different techniques and phospholipid systems. The beginnings of the two chains are conformationally and motionally nonequivalent; the *sn*-2 chain starts parallel to the interface while the *sn*-1 chain starts perpendicular and is less restricted in terms of rotamer states, as illustrated schematically in Figure 6 for mixed micelles. This has been shown in other phospholipid systems such as in crystals by X-ray diffraction (Hitchcock et al., 1974), in multibilayers by deuterium NMR and neutron diffraction (Oldfield et al., 1978; Seelig & Browning, 1978; Buldt et al., 1978), and in phospholipid micelles by ^1H and ^{13}C NMR (Roberts et al., 1978; Burns & Roberts, 1980). Monomeric phospholipids suggest a somewhat different model in which the distribution over rotational angles may be more uniform and the conformation of the two aliphatic chains is therefore not as highly differentiated (Roberts et al., 1978; Burns & Roberts, 1980).

Small unilamellar vesicles prepared by sonication constitute a convenient and widely used membrane model, and a similar phospholipid structure to mixed micelles is now suggested for these bilayers. Negatively charged vesicles where the signal was well resolved show a chemical shift difference of 0.07 ppm between the two α-methylene chains, which is somewhat less than that found with the same lipids in mixed micelles with Triton. Furthermore, the peaks of both chains are shifted downfield relative to the mixed micelles. With the aid of the enhancement technique, we were able to analyze quantitatively the fine structure of the signals. The *sn*-1 α-methylene protons appear as magnetically equivalent (A_2 system) while the *sn*-2 appears as magnetically nonequivalent (AB system). Moreover, the coupling constant with the β-methylene protons has about the same value for the two chains, $J_{\alpha\beta} = 7$ Hz. These results indicate that within the NMR time scale, free rotation of the *sn*-2 chain may not start until the β–methylene position while it already occurs at the α-methylene position for the *sn*-1 chain. This is in agreement with previous studies on PC/cholesterol multibilayers by ^2H NMR (Oldfield et al., 1978) and fits the neutron diffraction model in which the two chains are out of step by 1.5 C–C bond lengths (Zaccai et al., 1979). These results suggest further that the overall structure or conformation of the phospholipids at the interface does not depend on the particular aggregation state, i.e., crystals, micelles, multibilayers, or small unilamellar vesicles.

The results on vesicles made from PC are more difficult to interpret than those made from other phospholipids because of the broad α-methylene signal observed. This can be due to a smaller T_2 which is the consequence of more restricted motions and/or overlapping of several lines of slightly different chemical shifts. The peculiarity of PC may be related to that previously observed in mixed micelles of dipalmitoyl-PC and different kinds of detergents (Roberts et al., 1978). In nonionic detergent micelles with Triton X-100, the two peaks assigned to the *sn*-1 and *sn*-2 α-methylene groups could barely be separated; the use of enhancement methods was necessary to confirm the separation and observe the fine structure. On the other hand, dipalmitoyl-PC in ionic detergents gave sharp lines, and the fine structure of the signal was readily observed. The structures of the various detergents do differ from one another, and the effect of the detergent on the line width of the phospholipid may reflect a difference in the packing of the detergent at the interface, perhaps a tight packing with the nonionic Triton detergent and a looser one with the ionic ones because of the repulsion between groups of identical charge. Alternatively, this observation could be explained by greater chemical shift differences in the individual PC molecules in Triton micelles than in other detergents. In the case of ionic and zwitterionic phospholipid vesicles, it is possible that there is a looser packing in the negatively charged PA, PE, PS, and PG vesicles and a tighter one for zwitterionic PC vesicles. In this regard, titration experiments on monomolecular layers of PG show that there is a smaller surface area per molecule in the protonated state than in the ionic one (Sacre & Tocanne, 1977). On the other hand, a comparative study of PE, PS, and PC multibilayers by ^2H NMR showed that the choline and ethanolamine polar head groups are more freely rotating than the serine (Browning & Seelig, 1980). Of course, the packing of small unilamellar vesicles has been shown to be intrinsically different than in multibilayer structures (Sheetz & Chan, 1972; Bocian & Chan, 1978), but it would be surprising if the charge effect were different.

The broad α-methylene pattern in PC vesicles could also be due to a slightly different chemical shift between the inside and the outside layer of the vesicles. This feature has already been observed in the choline methyl groups by ^1H NMR (Kostelnik & Castellano, 1973; Eigenberg & Chan, 1980), the carbonyl groups by ^{13}C NMR (Schmidt et al., 1977), and the aliphatic chains at the C_7 and C_{12} positions by ^{19}F NMR (Longmuir & Dalquist, 1976) and is interpreted as a difference in the curvature of the two layers. The decoupled and enhanced α-methylene spectrum of egg PC vesicles shows three signals as do dilauryl- and dimyristoyl-PC vesicles. Further work is needed to understand the underlying cause of the

observed difference between zwitterionic and charged vesicles.

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References

Bocian, D. F., & Chan, S. (1978) *Annu. Rev. Chem. 29*, 307.
Browning, J. L., & Seelig, J. (1980) *Biochemistry 19*, 1262.
Buldt, G., Gally, H. U., Seelig, A., Seelig, J., & Zaccai, A. (1978) *Nature (London) 271*, 182.
Burns, R. A., & Roberts, M. F. (1980) *Biochemistry 19*, 3100.
Campbell, I. P., Dobson, C. M., Williams, R. J. P., & Xavier, A. V. (1973) *J. Magn. Reson. 11*, 172.
Clin, B., De Bony, J., Lalanne, P., Biais, J., Lemanceau, B. (1979) *J. Magn. Reson. 33*, 457.
De Marco, A., & Wüthrich, K. (1976) *J. Magn. Reson. 24*, 201.
Dennis, E. A., Ribeiro, A. A., Roberts, M. F., & Robson, R. J. (1979) *Solution Chem. Surfactants (Proc. Sect. 52nd Colloid Surf. Sci. Symp.) 1*, 175–194.
Eigenberg, K. E., & Chan, S. I. (1980) *Biochim. Biophys. Acta 599*, 330.
Folch, J. (1942) *J. Biol. Chem. 146*, 35.
Gaber, P. B., & Petitcolas, W. L. (1978) *Biophys. J. 24*, 677.
Gally, H. V., Pluschke, G., Overath, P., & Seelig, J. (1979) *Biochemistry 18*, 5605.
Gupta, C. M., Costello, C. E., & Khorana, H. G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3139.
Haberkorn, R. A., Griffin, R. G., Meadows, R. D., & Oldfield, E. (1977) *J. Am. Chem. Soc. 99*, 7353.
Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3036.
Kostelnik, R. J., & Castellano, S. (1973) *J. Magn. Reson. 9*, 291.
Longmuir, K. J., & Dahlquist, F. W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2716.
Oldfield, E., Meadows, M., Rice, D., & Jacobs, R. (1978) *Biochemistry 17*, 2727.
Pearson, R. H., & Pasher, I. (1979) *Nature (London) 281*, 499.
Rathbone, L., Magee, W. L., & Thompson, R. H. S. (1962) *Biochem. J. 83*, 498.
Ribeiro, A. A., & Dennis, E. A. (1975) *Biochemistry, 14*, 3746.
Roberts, M. F., & Dennis, E. A. (1977) *J. Am. Chem. Soc. 99*, 6142.
Roberts, M. F., Bothner-By, A. A., & Dennis, E. A. (1978) *Biochemistry 17*, 935.
Sacre, R. R., & Tocanne, J. F. (1977) *Chem. Phys. Lipids 18*, 334.
Schmidt, C. F., Barenholz, Y., Huang, C., & Thompson, T. E. (1977) *Biochemistry 16*, 3948.
Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839.
Seelig, A., & Seelig, J. (1975) *Biochim. Biophys. Acta 406*, 1.
Seelig, J., & Browning, J. L. (1978) *FEBS Lett. 92*, 41.
Seelig, J., Dijkman, R., & de Haas, G. H. (1980) *Biochemistry 19*, 2215.
Seiter, C. H. A., & Chan, S. I. (1973) *J. Am. Chem. Soc. 95*, 7561.
Sheetz, M. P., & Chan, S. I. (1972) *Biochemistry 11*, 4573.
Singleton, W. S., Gray, M. S., Brown, M. L., & White, J. L. (1965) *J. Am. Oil Chem. Soc. 42*, 53.
Stockton, G. W., Polnqszek, C. F., Leitch, L. C., Tulloch, A. P., and Smith, I. C. P. (1974) *Biochem. Biophys. Res. Commun. 60*, 844.
Stockton, G. W., Polnqszek, C. F., Tulloch, A. P., Hasan, F., & Smith, I. C. P. (1976) *Biochemistry, 15*, 954.
Zaccai, G., Buldt, G., & Seelig, J. (1979) *J. Mol. Biol. 134*, 693.