Biochimica et Biophysica Acta 856 (1986) 144–154
Elsevier

BBA 73000

Conformation of fatty acyl chains in α- and β-phosphatidylcholine and phosphatidylethanolamine derivatives in sonicated vesicles

Andreas Plückthun, Jacqueline DeBony, Tahsin Fanni and Edward A. Dennis *

Department of Chemistry, University of California at San Diego, La Jolla, CA 92093 (U.S.A.)

(Received September 30th, 1985)

Key words: Phospholipid; Methylated phospholipid; Acyl chain conformation; ^{1}H-NMR

Mono- and dimethylated derivatives constitute important intermediates in the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) in eucaryote membranes. ^{1}H-NMR techniques were utilized to examine the conformation of the region of the fatty acyl chains that is close to the polar group in the series of α-phospholipids: PE, *N*-methyl-PE, *N*,*N*-dimethyl-PE, and PC. The same series of polar groups, but on phospholipid containing *sn*-1 and/or *sn*-3 fatty acyl chains (β-phospholipids) were also examined. All of the phospholipids were in the form of small sonicated vesicles which are widely utilized as membrane models. The α-methylene group of the *sn*-1 and *sn*-2 fatty acyl chains of the α-phospholipids give rise to separate signals due to the non-equivalency of these chains with respect to the glycerol phosphate backbone on all α-phospholipids tested. Additionally, differences in the environment of the PC molecules as well as *N*-methyl-PE, and *N*,*N*-dimethyl-PE, but not PE itself on the inside and outside of the vesicles are reflected in the chemical shift of the α-methylene protons. On the other hand, all of the β-phospholipids (including β-PE) were found to reflect the inside/outside packing differences in their α-methylene groups. The bilayer packing does not induce any nonequivalence in the chemically equivalent acyl chains. In mixed micelles with detergents, β-phospholipids showed one α-CH_2 signal for all phospholipids. These results are consistent with a common conformational arrangement for the fatty acyl chains in all α-phospholipids that have been investigated no matter what aggregated form. The conformational arrangement in the β-phospholipids is different, but again is similar for all of the compounds tested in various aggregated forms.

Introduction

NMR studies utilizing various nuclei have proven extremely useful in elucidating phospholipid conformations in aggregated states. In particular, the nonequivalence of both fatty acyl chains of a variety of phospholipids in micelles of a large number of detergents and in sonicated vesicles of anionic phospholipids has been shown using ^{1}H-NMR [1–3]. These studies suggested that the conformational details of the phospholipid in these systems is similar to that originally observed in the crystal structure of both dilauroyl PE [4,5] and dimyristoyl PC [6]. Studies with ^{2}H-NMR were also found to be consistent with a nonequivalence of the fatty acyl groups in multibilayers found in the crystal structures [7–12]. Intermolecular cross-linking experiments [13], Raman spectroscopy [14], and neutron diffraction studies on specifically deuterated PC [15,16] also support the nonequivalence in packing of the two fatty acyl chains.

* To whom to address correspondence.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; SDS, sodium dodecyl sulfate; DTAPS, *N*,*N*-dimethyl-*N*-tetradecyl-3-ammoniopropane-1-sulfonate.

The methylated derivatives of PE have received a great deal of recent attention because as intermediates in the conversion of PE to PC via the methylation pathway, they have been suggested to play a critical role in membrane asymmetry and various activation events [17]. They also allow one to vary the polar group through a continuous series ranging from 0 to 3 methyl groups. The state of methylation also affects the susceptibility of the phospholipid to phospholipase A_2 catalyzed hydrolysis [18,19] and this may be reflected in conformational differences within the molecules. The β-phospholipids have also received recent attention as promising model membranes that may be somewhat less susceptible to degradation [20,21]. These compounds possess a plane of symmetry and therefore the structural non-equivalence observed in the fatty acyl chains of α-phospholipids is lacking.

Physical studies of β-phospholipids in multibilayers and in monolayers showed similarities to the corresponding α-phospholipids in many properties, such as the ability to form stable bilayers with the entrapment of water soluble molecules [20–23] and similar transition temperatures of the main transition [22]. Remarkable differences were, however, observed in area-pressure isotherms [10,24], indicating a more expanded state of β-PC than α-PC at all pressures, and in the absence of a subtransition in β-PC [25,26]. ^{2}H-NMR [10] and neutron diffraction [27,28] of β-PC in multibilayers have been interpreted to suggest an increased intermolecular chain-separation when compared with α-PC.

In this study, we have now compared the detailed packing of various phospholipids in small unilamellar vesicles and micelles. Small unilamellar vesicles constitute one of the most widely used model membrane systems. We wished to now question how the degree of methylation, ionic charge and position of the fatty acyl chain affect the conformation of the molecules as well as the difference in the packing of the inside and outside monolayers of the small vesicle. In addition, the fine structure of the α-methylene group in the ^{1}H-NMR spectrum of PC in vesicles could not previously be resolved and we have now investigated this question in more detail by comparing various α and β-phospholipids by using specifically deuterated PC and by using shift reagents.

A preliminary account of some of these studies was given at the 26th Annual Meeting of the Biophysical Society in Boston, MA, February, 1982.

Experimental section

Materials

Oleic acid, dipalmitoyl-α- and -β-PC, dipalmitoyl-*N*-methyl-α- and -β-PE, dipalmitoyl-*N*,*N*-dimethyl-α- and -β-PE, and dipalmitoyl-α- and -β-PE were obtained from Calbiochem. [2-^{2}H]Palmitic acid was obtained from Merck, Sharp and Dohme. 1-*O*-hexadecyl-2-palmitoyldeoxyamino-*sn*-glycero-3-phosphocholine was a generous gift from Dr. J. Hajdu, Boston College, Chestnut Hill, MA. All phospholipids gave single spots on TLC before use and only traces of hydrolysis products after the ^{1}H-NMR experiments. Lyso derivatives were prepared by treatment with cobra venom phospholipase A_2 [29] following procedures outlined elsewhere [30,31].

1-Palmitoyl-2-[2-$^2H_2$]palmitoyl-sn-glycero-3-phosphocholine. [2-$^2H_2$]Palmitic anhydride was prepared from [2-$^2H_2$]palmitic acid and DCCD in CCl_4 by the method of Selinger and Lapidot [32]. The reaction mixture was filtered, evaporated to dryness and employed in the acylation reaction directly. 1-Palmitoyl-*sn*-glycero-3-phosphocholine was prepared from 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine by treatment with phospholipase A_2 [30,31]. The lysophosphatidylcholine (1 mmol) was acylated with [2-$^2H_2$]palmitic anhydride (1 mmol) by the procedure of Gupta et al. [33] with the following modifications: Refluxing CH_2Cl_2 (25 ml) was used as solvent and *p*-pyrrolidinopyridine (1 mmol) as catalyst, and after completion of the reaction the mixture was diluted with 50 ml $CHCl_3/CH_3OH/H_2O$ (4 : 10 : 1, v/v), then passed over a mixed bed ion-exchange column (Rexyn I-300, 50 ml bed volume), which was washed with 100 ml of the same solvent. The eluant was then taken to dryness by repeated evaporation from benzene and purified by silicic acid chromatography with a $CHCl_3/CH_3OH$ step gradient. ^{1}H-NMR in C^2HCl_3 indicated that the signal from the *sn*-2 α-CH_2 group was reduced to 5–10% of the *sn*-1 signal. The remaining proton

signal in the *sn*-2 position is to be expected due to acyl migration during the experimental conditions utilized for acylation [31]. The deuterated fatty acid used in the synthesis did not have a detectable proton contamination.

1-[2-$^2H_2$]Palmitoyl-2-palmitoyl-sn-glycero-3-phosphocholine. 1,2-[2-$^2H_2$]Dipalmitoyl-*sn*-glycero-3-phosphocholine was prepared from *sn*-glycero-3-phosphocholine ($CdCl_2$ adduct, 0.5 mmol) and [2-$^2H_2$]palmitic anhydride (1.5 mmol) by the method of Patel et al. [34] and purified by silicic acid chromatography. The product was treated with phospholipase A_2 [30,31] and the reaction mixture was extracted three times with $CHCl_3/CH_3OH$ (2:1, v/v). The combined extracts were taken to dryness and used for acylation directly. The reacylation of 1-[2-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine with 5 mmol of palmitic anhydride was carried out as in the acylation of the lysoPC above. ^{1}H-NMR in C^2HCl_3 indicated that the signal from the *sn*-1 α-CH_2 was reduced to 5–10% of the *sn*-2 signal; the remaining protons are expected due to acyl migration as discussed above for the *sn*-2-labeled compound.

Vesicle preparation. About 20 mg of dry phospholipid was dispersed in 2 ml of ^2H_2O and the p^{2}H was adjusted as indicated. If the phospholipids were saturated, the dispersion was sonicated at about 60–70°C under argon with an MSE sonicator until the solution turned clear. This temperature should ensure full hydration in all cases [35–37]. The solution was then immediately transferred into the preheated NMR tube and kept in a sandbath at 60–70°C until the spectra were taken. If allowed to cool to room temperature, preparations of *N*-methyl-PE or *N*,*N*-dimethyl-PE would visibly aggregate within minutes. Dispersions of unsaturated phospholipids were sonicated at 0–5°C under argon. The p^{2}H was checked and adjusted if necessary.

When centrifuged vesicles were used, the following procedure was employed: The vesicles were sonicated as above and centrifuged in a Beckman L2-65B ultracentrifuge at $100\,000 \times g$ for 30 min at room temperature. Multibilayers float on the ^2H_2O solution. This top layer was carefully removed with a Pasteur pipette and the residual clear solution transferred to an NMR tube. This tube was then incubated at about 10 Cdeg above the phase transition temperature of the phospholipid in an oven or a sandbath for about 1 h to achieve 'annealing' of the vesicles [38]. Vesicles of β-PC with $MnCl_2$ either inside or outside were prepared by the method of Schuh et al. [39].

Sonicated vesicles of dipalmitoyl-α-PC and dipalmitoyl-β-PC were subjected to gel permeation chromatography on a Sepharose CL-4B column at 50°C as described elsewhere [40]. Both were found to elute at an almost identical volume which was, furthermore, identical with experimental error to the elution volume of egg PC vesicles.

^{1}H-NMR spectroscopy. ^{1}H-NMR experiments were performed at 360 MHz on a modified Varian Instrument with an Oxford Instruments magnet and a Nicolet computer. The quadrature phase detection was used and the spectral width was 2000 Hz. The memory was 16K and 200–500 scans were accumulated, using a delay time of 2 s. Usually, a line broadening of 1 Hz was used. TSP was included in all samples as a chemical shift standard. All spectra were obtained at 65°C, unless otherwise indicated. Resolution enhancement was carried out as described previously [3,41]. Computer simulations were carried out on the Nicolet computer with the NFTSIM program. Preliminary experiments were also carried out at 500 MHz (where indicated) at the Regional NMR Facility at the California Institute of Technology, Pasadena, CA.

^{31}P-NMR. ^{31}P-NMR experiments were carried out at 40.3 MHz on a modified JEOL-PFT-100 system equipped with a modified Nicolet 1080 computer and disk.

Results

Phosphatidylcholine vesicles

The ^{1}H-NMR spectrum of sonicated vesicles of dipalmitoyl PC is shown in Fig. 1. The α-CH_2 region with and without β-CH_2 decoupling as well as with and without resolution enhancement is expanded in the figure. These spectra are sharply distinguished from those obtained previously from micelles, mixed micelles or vesicles of anionic phospholipids [1–3] by the existence of three main resonances at about 2.26 ppm, 2.34 ppm and 2.30 ppm. When extreme line broadening (10–20 Hz) was applied to a computer simulation using previ-

ous data for chemical shifts and coupling constants, there were still only two peaks observed, clearly corresponding to the *sn*-1 and *sn*-2 α-CH_2 groups. This result was not changed by varying the γ_A-γ_B chemical shift difference of the AB-quartet or by varying the *sn*-1-*sn*-2 chemical shift difference or the coupling constants within the values that have been previously observed for anionic and zwitterionic phospholipids [2,3]. The spectrum of PC in vesicles is different from anionic phospholipids both in the shape of the resonances around 2.3 ppm and in their broader linewidth. The spectra of anionic phospholipids in vesicles can be resolved by using resolution enhancement to give a fine structure which is essentially identical to that observed for micelles [3]. For vesicles of dipalmitoyl PC, no significant improvement in resolution was obtained at 500 MHz as opposed to 360 MHz; nor did the removal of aggregated material by centrifugation change the spectrum obtained (data not shown).

Since the resolution enhancement may substantially distort the relative intensities of the peaks, the resonances of small amounts of impurities or hydrolysis products might, under certain circumstances, be amplified out of proportion. As a control, several impurities were therefore added to

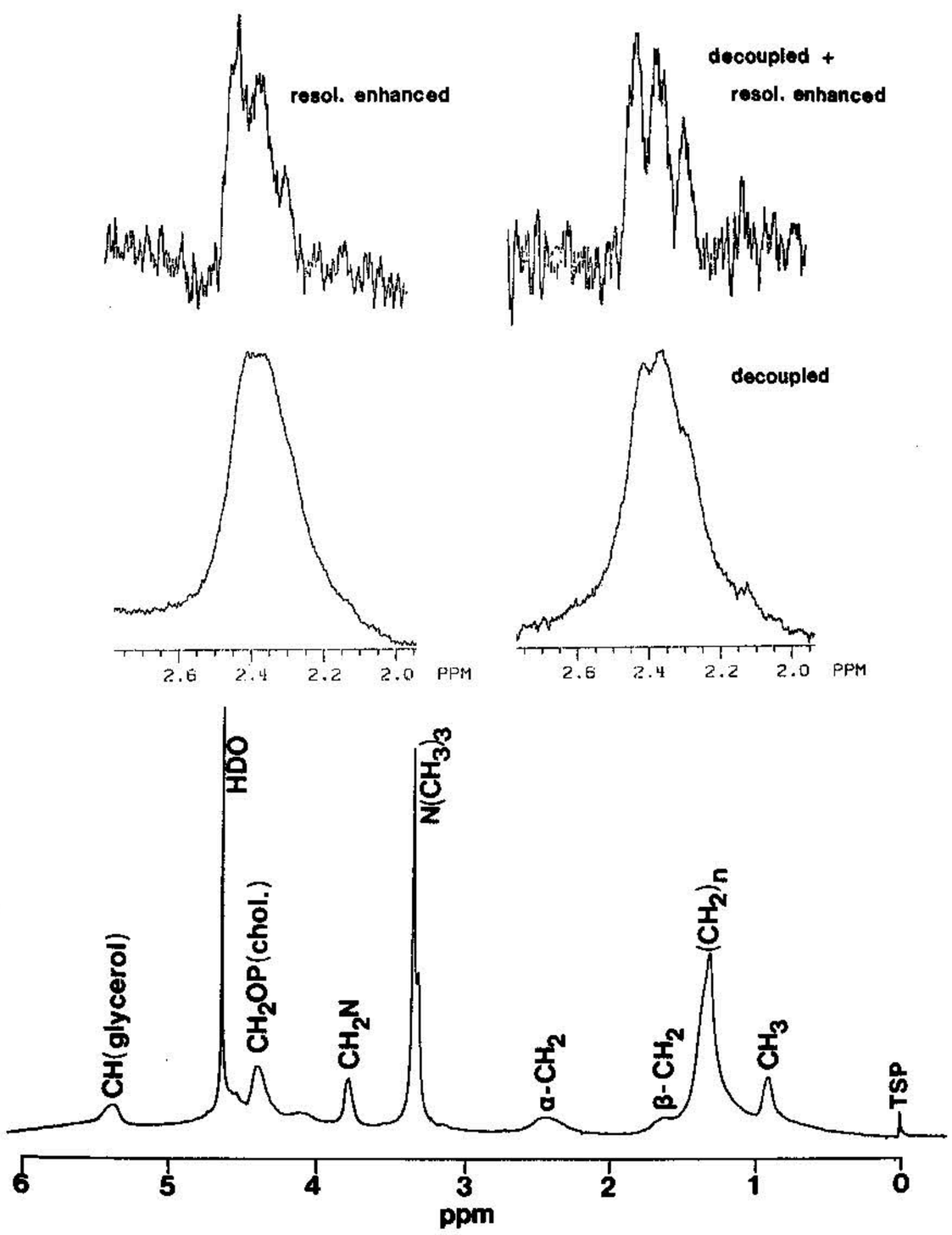

Fig. 1. ^{1}H-NMR spectra of sonicated vesicles of dipalmitoyl PC. On the bottom, the full spectrum is shown. On the top, the expanded α-methylene region is shown, with and without β-methylene decoupling as well as with and without resolution enhancement, as indicated in the figure.

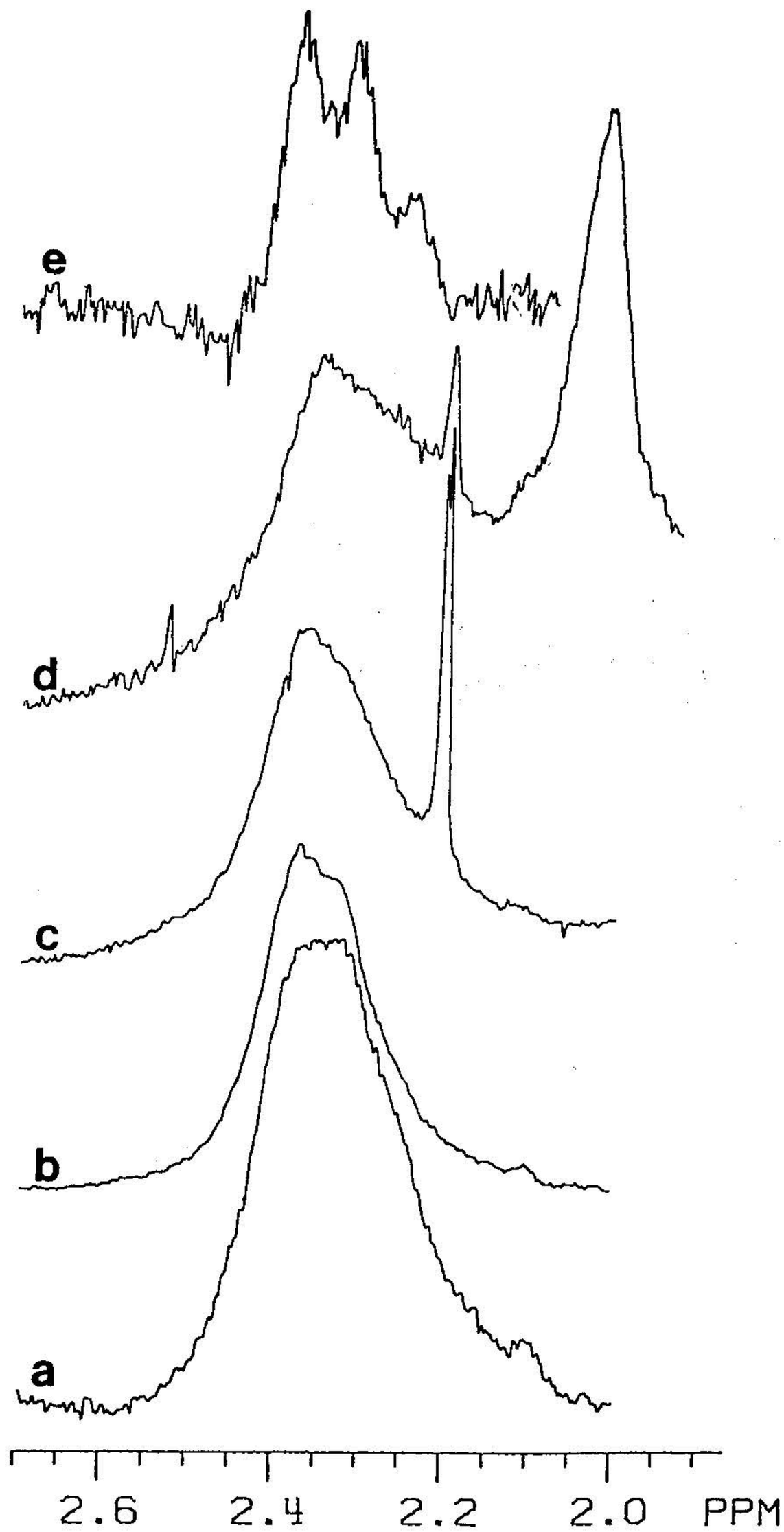

Fig. 2. ^{1}H-NMR spectra of the 2.0–2.6 ppm region of sonicated vesicles of dipalmitoyl PC, cosonicated with various additions: (a) no addition, (b) with 20 mol% β-dipalmitoyl PC, (c) with 20 mol% palmitoyl lysoPC, (d) with 20 mol% oleic acid, (e) no addition, but β-methylene decoupled and resolution enhanced. The peak at 2.0 ppm in (d) is due to the methylene groups adjacent to the double bond in oleic acid.

the lipid before sonication and the mixture was cosonicated to achieve as much incorporation into the vesicle as possible. Neither the addition of 20 mol% oleic acid nor the addition of 20 mol% 1-palmitoyl lysoPC dramatically changed intensities of the peaks in question but rather gave rise to new resonances at higher field (Fig. 2), possibly caused by nonincorporated fatty acid or lysoPC. The addition of phospholipase A_2 to the vesicle preparation leads to an in situ generation of lysoPC and fatty acid, but this enzymatic hydrolysis did not measurably change the resonances in question; rather, the fine structure was no longer visible (data not shown). There is no evidence, either, that PC is hydrolyzed faster in water than other phospholipids [42]. The resonance of β-PC is identical to the downfield peak at 2.39 ppm. Therefore, ^{31}P-NMR chemical shifts were determined (Table I) to show that α- and β-phospholipids as well as their lyso products can be clearly distinguished by this technique. The commercial phospholipids used in this study did not show any evidence for the presence of β-phospholipids (detection limit: about 1%). Furthermore, such an impurity cannot give rise to the observed phenomenon of three main resonances, since the same type of spectrum is observed in vesicles from natural egg PC [3] which contains no β-PC, as in vesicles from synthetic PC.

TABLE I

^{31}P-NMR CHEMICAL SHIFTS OF PHOSPHOLIPIDS (6 mM) IN THE PRESENCE OF TRITON X-100 (48 mM)

Phospholipid	Chemical shift (p.p.m.) [a]
α-PE	0.15
α-LysoPE	−0.26
N-Methyl-α-PE	0.30
N-Methyl-α-lysoPE	−0.10
N,*N*-Dimethyl-α-PE	0.42
N,*N*-Dimethyl-α-LysoPE	0.02
α-PC	0.86
α-LysoPC	0.37
β-PE	0.71
β-LysoPE	0.46
N-Methyl-β-PE	0.86
N-Methyl-β-lysoPE	0.60
N,*N*-Dimethyl-β-PE	1.02
N,*N*-Dimethyl-β-lysoPE	0.75
β-PC	1.45
β-LysoPC	1.13

[a] Relative to H_3PO_4 (85%) upfield is positive, temperature 40°C.

From these control experiments, it appears unlikely that the peculiar spectrum of PC in vesicles is due to the presence of either hydrolysis products or isomeric impurities.

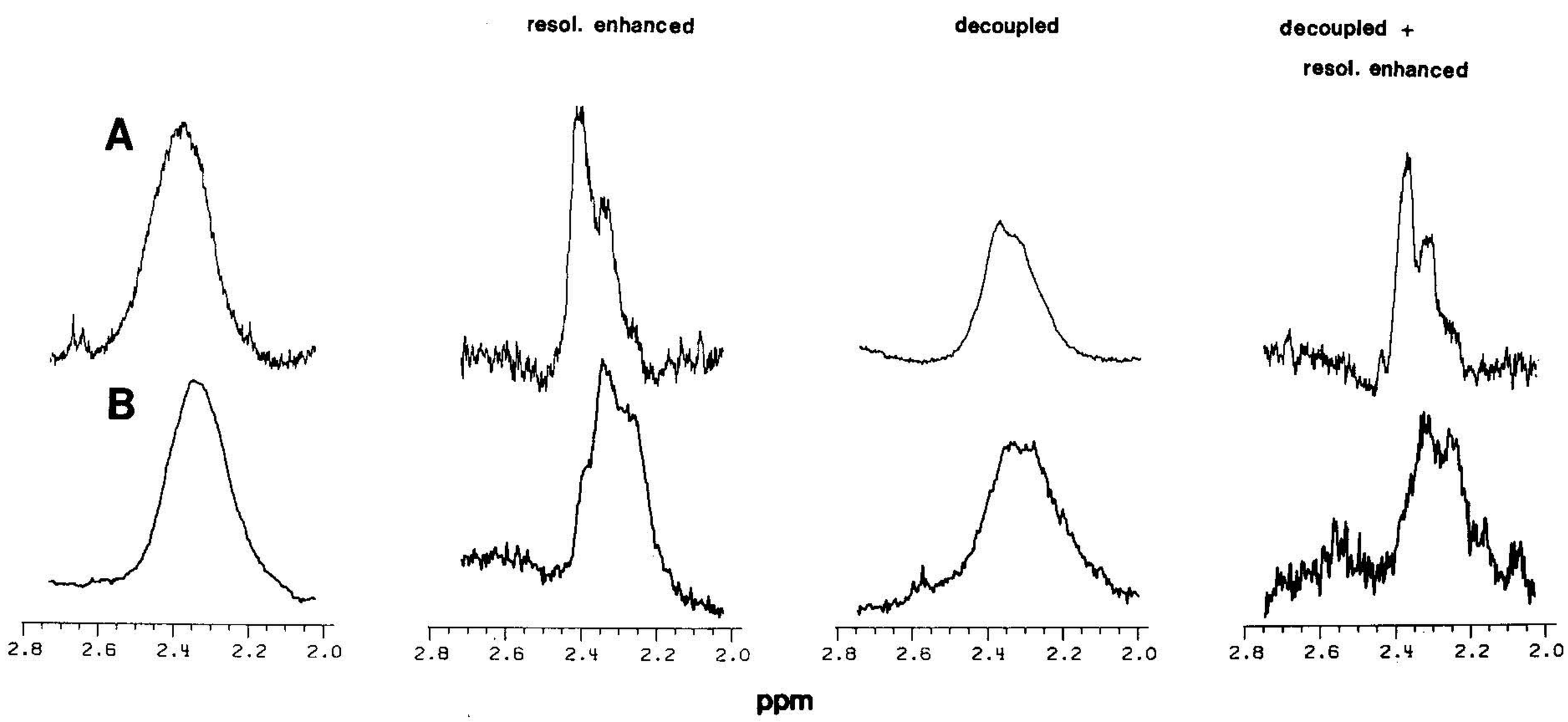

Fig. 3. ^{1}H-NMR spectra of the α-methylene region of sonicated vesicles of specifically deuterated dipalmitoyl PC, (A) deuterated in the α-methylene group of the *sn*-1 position, (B) deuterated in the α-methylene group of the *sn*-2 position. Spectra with and without decoupling, as well as with and without resolution enhancement are shown for each compound.

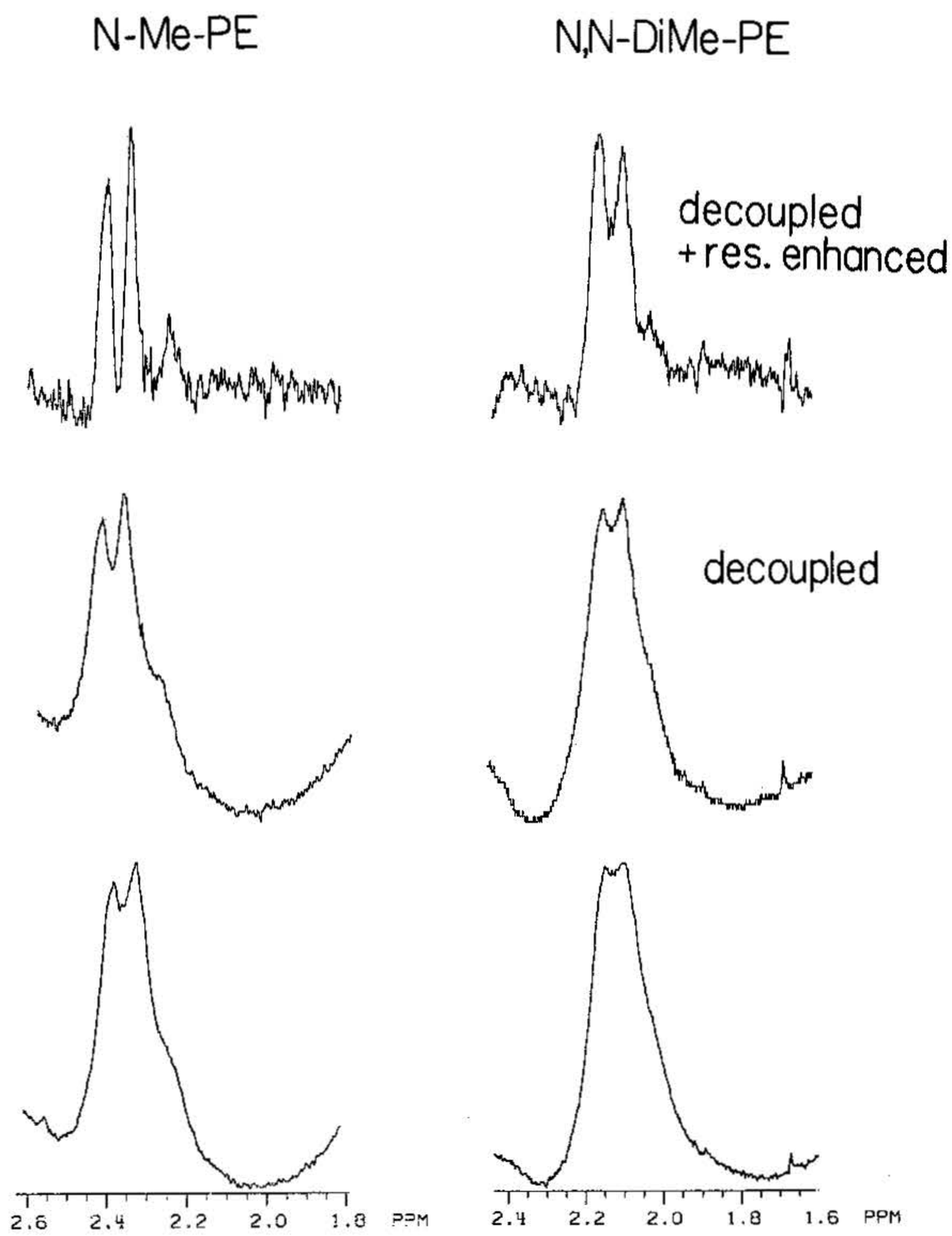

Fig. 4. ^{1}H-NMR spectra of the α-methylene region of sonicated vesicles of *N*-methyl-PE and *N*,*N*-dimethyl-PE at p^{2}H 10.6. Spectra with and without β-methylene decoupling as well as with decoupling and resolution enhancement are shown.

^{1}H-NMR spectra were then obtained from vesicles of the specifically deuterated phospholipids 1-palmitoyl-2-[2-$^2H_2$]palmitoyl-*sn*-glycero-3-phosphocholine and 1-[2-$^2H_2$]-palmitoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (Fig. 3). The linewidth still remained too broad to resolve a fine structure. It must be noted that neither of the two compounds gave rise to a symmetrical peak but rather produced a shoulder on the upfield side. This is reminiscent of the $N(CH_3)_3$ signal, where this asymmetry has been assigned to resonances from the outside and inside of the vesicle [43]. The addition of the shift reagent $PrCl_3$ caused too much further broadening to give an improvement in separation of the hypothetical inside and outside signals. A comparison of these results to large unilamellar vesicles, where there is no difference in curvature and packing between the inside and outside of the vesicle, cannot be carried out in practice, since linewidths in the latter are even larger. The reasons for this have been discussed in detail by Bocian and Chan [44].

The presence of inside-outside peaks was further confirmed by adding the broadening reagent $MnCl_2$ to the inside or outside of vesicles of α-PC [39]. The center of the outside peak was around 2.38 ppm whereas the center of the inside peak was around 2.26 ppm. The outside signal could be resolved with resolution enhancement techniques into two very broad peaks, presumably corresponding to *sn*-1 and *sn*-2 signals. No fine structure, however, could be resolved. The inside signal is so broad that not even the *sn*-1 and *sn*-2 signals can be resolved. These conclusions are consistent with similar observations by Chan and co-workers [39], who attribute the different linewidth in the inside and outside signal to different packing geometries (see below).

α-Phosphatidylethanolamine derivatives in vesicles

The ^{1}H-NMR spectra of *N*-methyl-PE and *N*,*N*-dimethyl-PE (Fig. 4) show a pattern similar to PC. PE has been previously demonstrated to be resolvable to its expected fine structure of the α-CH_2 group [3]. Spectra at various pH values from far below to slightly above the pK_a of the

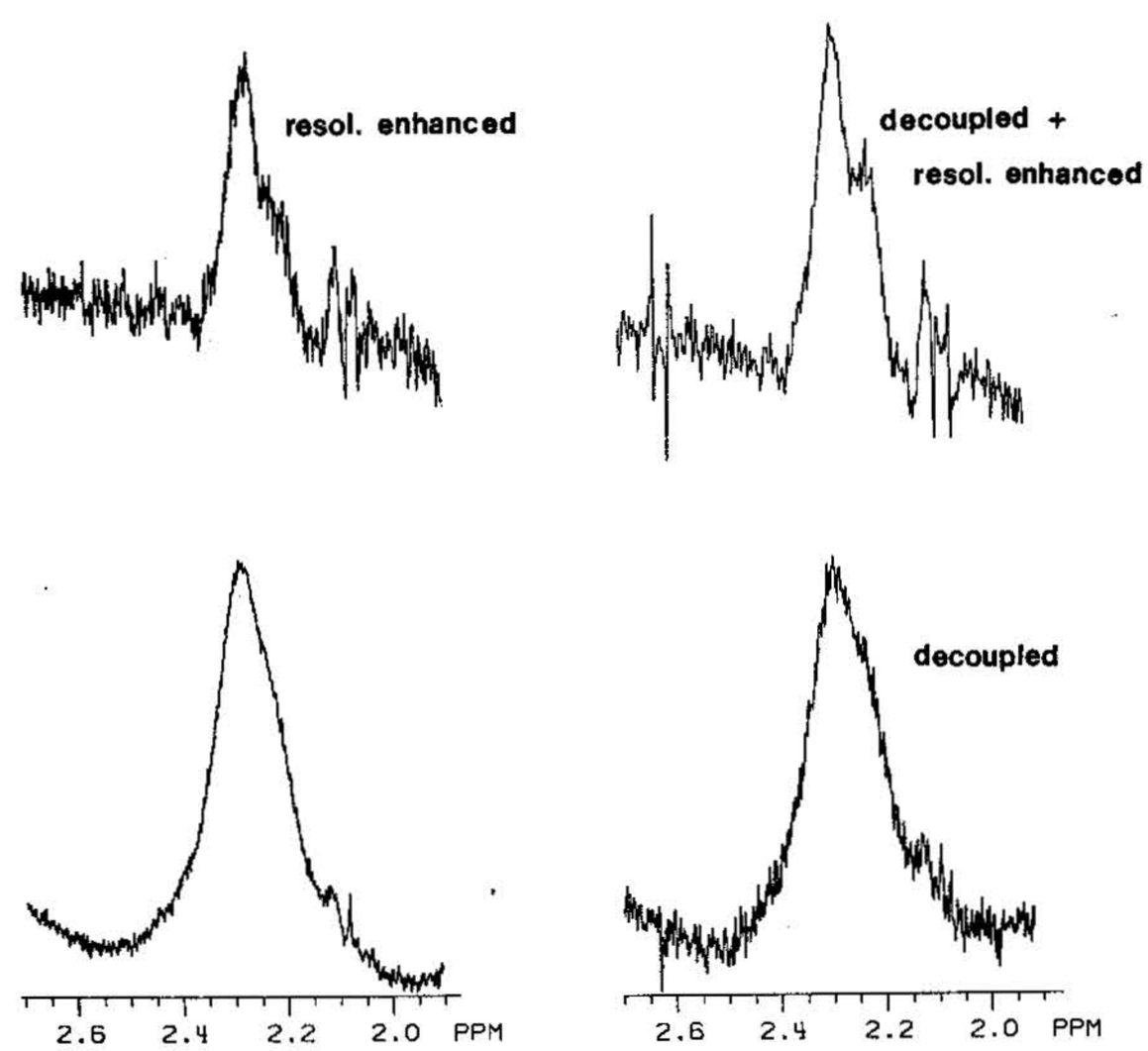

Fig. 5. ^{1}H-NMR spectra of the α-methylene region of sonicated vesicles of 1-*O*-hexadecyl-2-palmitoyldeoxyamino-*sn*-glycero-3-phosphocholine. Spectra with and without β-methylene decoupling as well as with and without resolution enhancement are shown.

amino group were different in linewidth (with the negatively charged phospholipid giving the sharpest signal), but were all identical in showing three main resonances. The fact that the appearance of the signal does not noticeably change with time over several hours makes it again unlikely that the three peaks are due to hydrolysis. Only about 5% hydrolysis has been observed and only about 5% hydrolysis would be expected in 3 h at pH 11.0 [42], but no change in the spectrum with time was observed.

In a further attempt to test the hypothesis that the more complicated pattern around 2.3 ppm arises from chemical shift differences between the inside and outside of the vesicles in addition to differences between the *sn*-1 and *sn*-2 position, spectra of other phospholipid vesicles were obtained that do not have a fatty acid linked to the *sn*-1 position and have, therefore, only one α-CH_2 group. The single α-CH_2 group of sphingomyelin (of the fatty acid linked by an amide bond) could not be resolved into any fine structure in vesicles (data not shown). However, the synthetic phospholipid 1-hexadecyl-2-palmitoyldeoxyamino-*sn*-glycero-3-phosphocholine, which also contains only one α-CH_2 group, gave rise to a spectrum which could be resolved into two main peaks, with the upfield one (presumably from the inside of the vesicle) being the smaller one (Fig. 5).

β-Phospholipids

β-Phospholipids have a plane of symmetry and therefore no asymmetric center. They can give rise, therefore, to only one signal for the α-CH_2 groups of both chains. This is shown in Fig. 6 for β-PC in several detergents. In vesicles, however, all β-phospholipids tested showed an additional upfield peak with an intensity ratio of about 2 : 1 (Fig. 7). This intensity ratio can be estimated without the use of resolution enhancement and is therefore not distorted. These data suggest that an inside-outside difference can indeed by observed in this part of the phospholipid molecule. This is confirmed by Mn^{2+} broadening experiments on β-phosphatidylcholine as shown in Fig. 8.

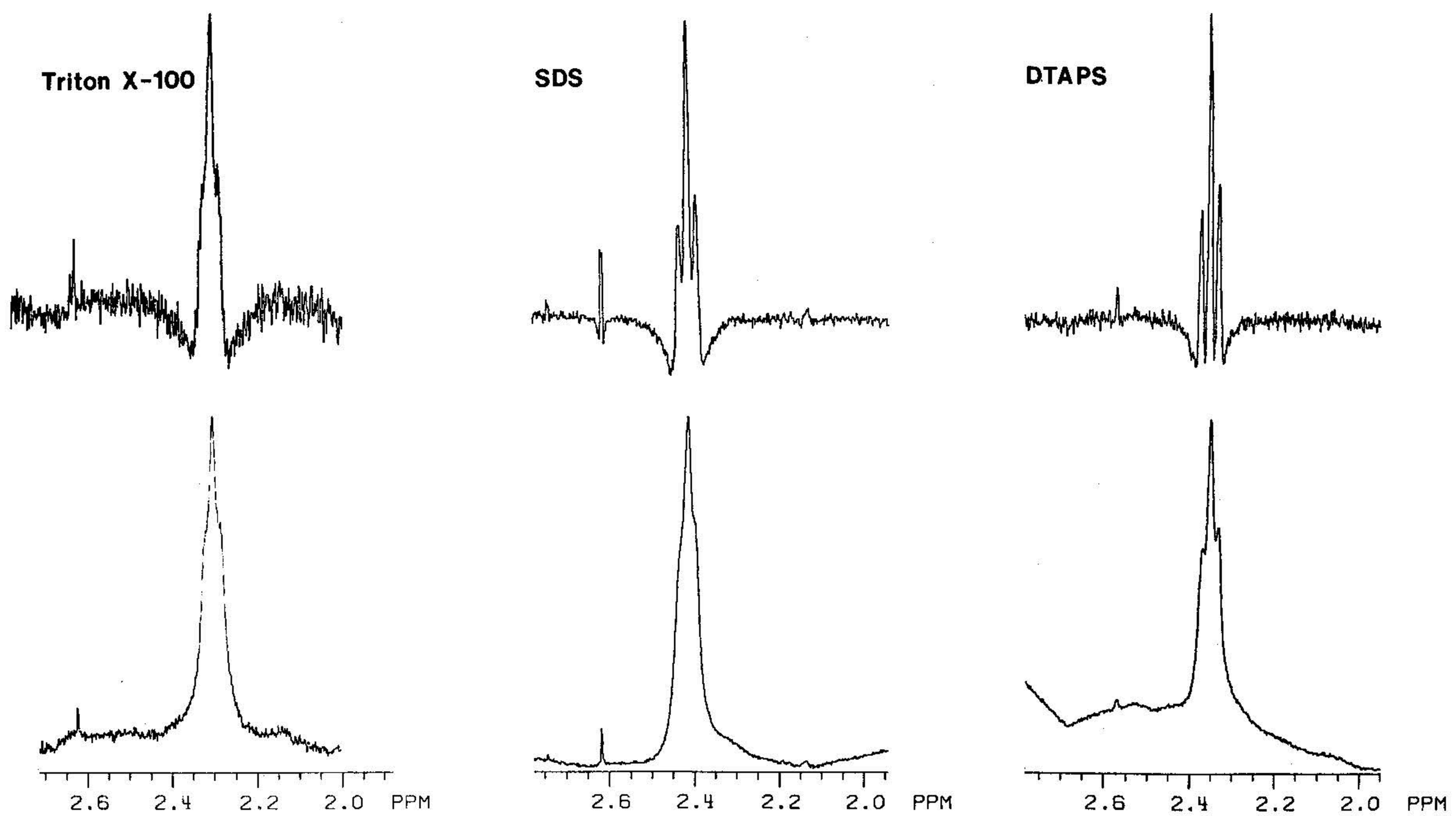

Fig. 6. ^{1}H-NMR spectra of the α-methylene region of β-dipalmitoyl PC in mixed micelles with Triton X-100, sodium dodecylsulfate (SDS) and *N*,*N*-dimethyl-*N*-tetradecyl-3-ammoniopropane-1-sulfonate (DTAPS). The detergent to phospholipid ratio was 8.1 in all cases. The spectra in the top row are with resolution enhancement, while the spectra in the bottom row are not. All spectra were obtained at 40°C.

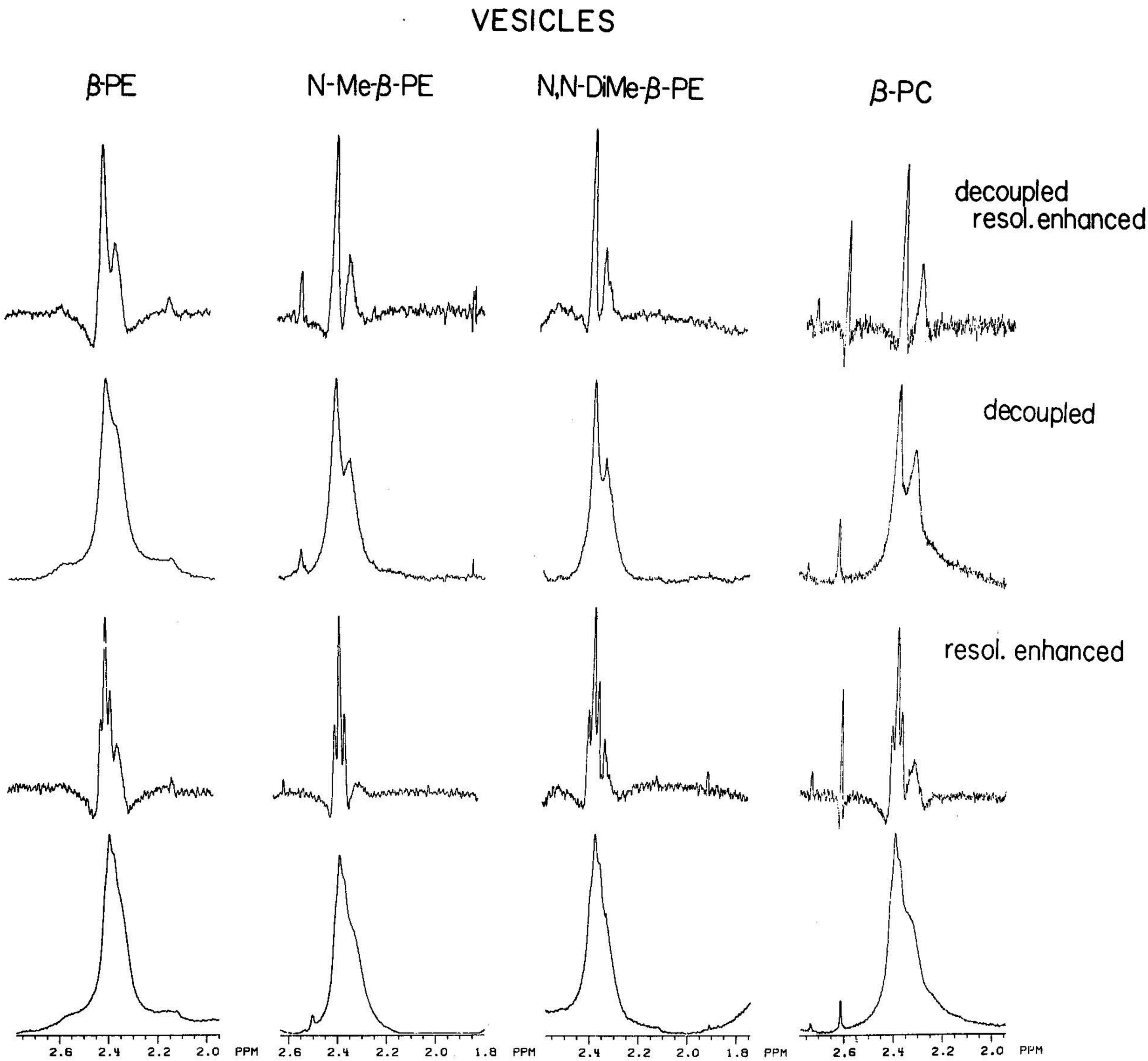

Fig. 7. ^{1}H-NMR spectra of the α-methylene region of sonicated vesicles of β-PE, *N*-methyl-β-PE, *N*,*N*-dimethyl-β-PE, and β-PC. Spectra with and without β-methylene decoupling as well as with and without resolution enhancement are shown, as indicated in the figure.

Discussion

The results reported here demonstrate that ^{1}H-NMR can detect spectral differences in the β-phospholipids as well as in some α-phospholipids between the inside and outside monolayer of the vesicle. Fig. 7 indicates a trend towards a larger shift-difference between the inside and outside monolayer of β-phospholipids with an increasing number of methyl groups. In α-phospholipids, this trend is very much more difficult to follow because of the additional signals arising from the intramolecular non-equivalence of the acyl chains. Qualitatively, one can observe that anionic phospholipids (such as PE at high pH) give a pattern for the α-CH_2 region in vesicles which can be completely simulated with only one signal for the *sn*-1 α-methylene and another one for the *sn*-2 α-methyl-

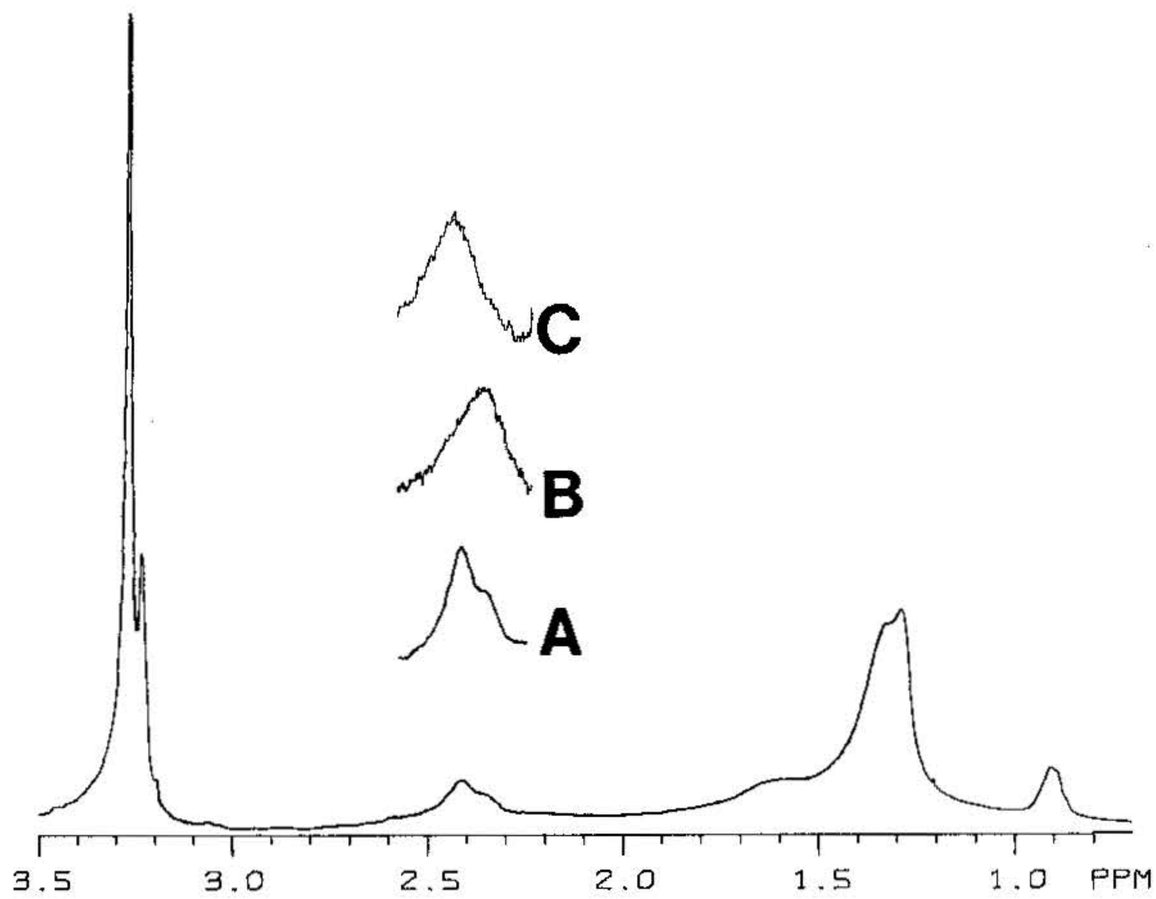

Fig. 8. ^{1}H-NMR spectra of sonicated vesicles of β-PC. On the bottom, the full spectrum is shown. The inserts show the α-methylene region of vesicles (A) without Mn^{2+} added, (B) with Mn^{2+} on the outside and (C) with Mn^{2+} on the inside. All spectra were taken at 40°C without decoupling or resolution enhancement. The chemical shift of all inserts is relative to the terminal methyl protons on the fatty acyl group.

ene group [3]; therefore, the inside and outside monolayers are apparently not resolved. In partially or completely zwitterionic phospholipids with 1–3 methyl groups on the nitrogen, such an inside-outside difference becomes observable. We have shown here by selective deuteration and shift reagents that the complicated α-CH_2 region of methylated α-PE derivatives and α-PC is due to different signals from the *sn*-1 and *sn*-2 as well as inside and outside of the vesicle.

The two monolayers of the bilayer in small sonicated vesicles exhibit curvatures which differ in both magnitude and size. Previous studies on specifically fluorinated PC vesicles with ^{19}F-NMR have shown differences in both chemical shift and linewidth for the inside and outside of the vesicle [45]. The differences in chemical shift of the *N*-methyl protons from the inside and outside of the vesicle as well as their different relaxation times are now well established [46,47]. Chan and co-workers [39] showed by magnetization transfer experiments that the acyl chains on the inside of the vesicle give rise to a sharp component in the bulk methylene signal, while the outer acyl chains give rise to a broad component. This is consistent with the view that there is a greater degree of motional restriction for the fatty acids of the outer monolayer. The packing density of the phospholipids in the inner monolayer is determined by the distance between the head groups, leaving more room for the fatty acids. It is therefore not surprising that this difference in packing and environment manifests itself in the chemical shift differences of the α-methylene groups, as suggested by the studies described here. Apparently, charge repulsion between anionic headgroups may alleviate the difference between the two monolayers somewhat, at least around the carbonyl group region. In addition, non-methylated α-PE may have less steric hindrance between the small headgroups so that the spacing of the fatty acids in the inner monolayer is as tight as in the outer [48,49].

While the more extended intramolecular chain-spacing in β-phospholipids may make all head group contacts less significant and therefore lead to observable inside/outside packing differences for PE and all methylated derivatives, an increase in separation between the inside and outside signal is still apparent with an increasing number of methyl groups, presumably due to the same factors as in the α-phospholipids.

Our results are consistent with a conformational arrangement of the fatty acids relative to the interface that is the same in all aggregates of α-phospholipids. In methylated PE derivatives and PC, however, the signals are more difficult to assign. The difference in chemical shift between the A and B proton of the *sn*-2 α-CH_2 group is remarkably constant for natural phospholipids both in vesicles and in micelles with Triton X-100 [3]. It does become smaller, however, with shorter chain lengths of the phospholipid and finally zero when the phospholipid is monomerically dispersed. It had been suggested that it also becomes rather small in certain anionic detergents [2]. Relaxation studies [44,50,51] suggest that a rapid, limited torsional motion of several bonds, which may also involve the bonds adjacent to the α-CH_2 group of the fatty acids, can occur in aggregated phospholipids. A complete rotation of the CO-$CH_2(\alpha)$ bond in the *sn*-2 chain would require the fatty acid to swing significantly away from the *sn*-1 fatty acid. This is presumably not possible in a phospholipid aggregate for both kinetic and thermodynamic reasons. The population of the

'aggregate-forbidden' rotamer(s) probably increases with shorter chain length until in predominantly monomeric phospholipids or in aggregates with a very loose packing, the population of the different rotamers is such that the chemical shift of both the A proton and the B proton average out to the same value.

The ^{1}H-NMR experiments do not necessarily allow the conclusion that rotations of the α-CH_2 group in the *sn*-2 position are completely frozen, but only that the population of rotamers is different in a monomeric phospholipid from that in a micellar one. ^{2}H-NMR studies in which only one of the two hydrogens in the α-methylene group of the *sn*-2 chain was deuterated also support the idea that the motions of the two hydrogens of the *sn*-2 chain are different [52]. The ^{1}H-NMR experiments are consistent with a rapid motion of the α-CH_2 group of the *sn*-1 chain, but cannot prove it. An AB-quartet has so far never been observed in this resonance, and it is therefore not clear whether the distance to the asymmetric center is too large to induce a chiral environment for the development of an AB-quartet, or whether the rotational motion is indeed unhindered. On the other hand, deuterium NMR has shown that the two deuterons in the α-C^2H_2 position of the *sn*-1 chain are motionally equivalent, suggesting indeed rapid averaging about an axis perpendicular to the HCH plane and through the carbon atom [53].

Becuase of the plane of symmetry in β-phospholipids, an identical conformation for both fatty acid chains might be expected in aggregates, but, strictly speaking, is not required. The ^{1}H-NMR spectra show that both chains give rise to only one inside triplet and one outside triplet. Since, however, the distance between the α-CH_2 group and the prochiral center might, as in the *sn*-1 position in α-phospholipids be too great to induce a chiral environment, the fine structure might not be diagnostic for the motion of the fatty acids. ^{2}H-NMR experiments in multibilayers [10] have shown that all four deuterons in both α-C^2H_2 positions of specifically deuterated β-PC give rise to only one quadrupolar splitting, also suggesting motional averaging. The similarity in size of this quadrupolar splitting to the one observed for the α-C^2H_2 group of the *sn*-2 position in α-PC prompted Seelig et al. [10] to propose that both chains are bent like the *sn*-2 chain in α-PC. Further support was obtained by neutron diffraction [27,28], and the various hydrated phases have now been analyzed by X-ray diffraction and differential scanning calorimetry [26]. Together, the studies suggest that the glycerol backbone of β-PC exists in a more extended conformation than in α-PC with a greater intramolecular distance between the two fatty acid chains and differences in the packing behavior of the two phospholipids. However, these methods do not lend themselves to studies in micelles and vesicles. With ^{1}H-NMR, these structures become accessible and we have extended the range of polar groups studied. We have shown here that conformations obtained from multibilayer studies are consistent with results obtained in micelles and sonicated vesicles for a variety of head groups in both α- and β-phospholipids. These results taken together suggest a remarkably similar backbone conformation for all α-phospholipids in any aggregate and a different one common to all β-phospholipids in all aggregated states.

Acknowledgements

This work was supported by NSF Grant DMB 85-18684. Experiments at 500 MHz were carried out at the Regional NMR Facility supported by the NSF (Grant CHE 79-16324).

References

1 Roberts, M.F. and Dennis, E.A. (1977) J. Am. Chem. Soc. 99, 6142
2 Roberts, M.F., Bothner-By, A.A. and Dennis, E.A. (1978) Biochemistry 17, 935
3 DeBony, J. and Dennis, E.A. (1981) Biochemistry 20, 5256
4 Hitchcock, P.B., Mason, R., Thomas, K.M. and Shipley, G.G. (1974) Proc. Natl. Acad. Sci. USA 71, 3036
5 Hitchcock, P.B., Mason, R. and Shipley, G.G. (1975) J. Mol. Biol. 94, 297
6 Pearson, R.H. and Pascher, I. (1979) Nature 281, 499
7 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839
8 Seelig, A. and Seelig, J. (1975) Biochim. Biophys. Acta 406, 1
9 Seelig, J. and Browning, J.L. (1978) FEBS Lett. 92, 41
10 Seelig, J., Dijkman, R. and De Haas, G.H. (1980) Biochemistry 19, 2215
11 Gally, H.U., Pluschke, G., Overah, P. and Seelig, J. (1979) Biochemistry 18, 5605
12 Browning, J.L. and Seelig, J. (1980) Biochemistry 19, 1262
13 Gupta, C.M., Costello, C.E. and Khorana, H.G. (1979) Proc. Natl. Acad. Sci. USA 76, 3139

14 Gaber, B.P., Yager, P. and Peticolas, W.L. (1978) Biophys. J. 24, 677
15 Büldt, G., Gally, H.U., Seelig, A., Seelig, J. and Zaccai, G. (1978) Nature 271, 182
16 Zaccai, G., Büldt, G., Seelig, A. and Seelig, J. (1979) J. Mol. Biol. 134, 693
17 Hirata, F. and Axelrod, J. (1980) Science 209, 1082
18 Adamich, M., Roberts, M.F. and Dennis, E.A. (1979) Biochemistry 18, 3308
19 Dennis, E.A. (1983) The Enzymes, Vol. 16, p. 307
20 Okahata, Y. and Lim, H.Y. (1984) J. Am. Chem. Soc. 106, 4696
21 Okahata, Y., Lim, H.Y., Hachiya, S. and Nakamuna, G. (1984) J. Membrane Sci. 19, 237
22 Kunitake, T., Okahata, Y. and Tawaki, S. (1985) J. Coll. Interface Sci. 103, 190
23 Smith, N.B. (1981) Chem. Phys. Lipids 29, 277
24 Llerenas, E. and Mingins, T. (1976) Biochim. Biophys. Acta 419, 381
25 Stümpel, J., Eibl, H. and Niksch, A. (1983) Biochim. Biophys. Acta 727, 246
26 Serrallach, E.N., Dijhman, R., De Haas, G.H. and Shipley, G.G. (1983) J. Mol. Biol. 170, 155
27 Büldt, G. and De Haas, G.H. (1982) J. Mol. Biol. 158, 55
28 Büldt, G. (1982) Basic Life Sci. (Neutrons Biol.) 27, 189
29 Deems, R.A. and Dennis, E.A. (1981) Methods Enzymol. 71, 703
30 Plückthun, A. and Dennis, E.A. (1981) J. Phys. Chem. 85, 678
31 Plückthun, A. and Dennis, E.A. (1982) Biochemistry 21, 1743
32 Selinger, Z. and Lapidot, Y. (1966) J. Lipid Res. 7, 174
33 Gupta, C.M., Radhakrishnan, R. and Khorana, H.G. (1977) Proc. Natl. Acad. Sci. USA 74, 4315
34 Patel, K.M., Morrisett, J.D. and Sparrow, J.T. (1979) J. Lipid Res. 20, 674
35 Mantsch, H.H., Hsi, S.C., Butler, K.W. and Cameron, D.G. (1983) Biochim. Biophys. Acta 728, 325
36 Chang, H. and Epand, R.M. (1983) Biochim. Biophys. Acta 728, 319
37 Seddon, J.M., Cerc, G. and Marsh, D. (1983) Biochemistry 22, 1280
38 Lawaczeck, R., Kainosho, M. and Chan, S.I. (1976) Biochim. Biophys. Acta 443, 313
39 Schuh, J.R., Banerjee, U., Muller, L. and Chan, S.I. (1982) Biochim. Biophys. Acta 687, 219
40 Kensil, C.R. and Dennis, E.A. (1979) J. Biol. Chem. 254, 5843
41 Clin, B., DeBony, J., Lalanne, P., Biais, J. and Lemanceau, B. (1979) J. Magn. Res. 33, 457
42 Kensil, C.R. and Dennis, E.A. (1981) Biochemistry 21, 6079
43 Bergelson, L.D. (1978) Methods Membrane Biol. 9, 275
44 Bocian, D.F. and Chan, S. (1978) Annu. Rev. Phys. Chem. 29, 307
45 Longmuir, K.J. and Dahlquist, F.W. (1976) Proc. Natl. Acad. Sci. USA 73, 2716
46 Kroon, P.A., Kainosho, M. and Chan, S.I. (1976) Biochim. Biophys. Acta 433, 282
47 Eigenberg, K.E. and Chan, S.I. (1980) Biochim. Biophys. Acta 599, 330
48 Hauser, H., Pascher, I., Pearson, R.H. and Sundell, S. (1981) Biochim. Biophys. Acta 650, 21
49 Marsh, D., Watts, A. and Smith, I.C.P. (1983) Biochemistry 22, 3023
50 Gent, M.P.N. and Prestegard, J.H. (1977) J. Magn. Res. 25, 243
51 Fuson, M.M. and Prestegard, J.H. (1983) Biochemistry 22, 1311
52 Engel, A.K. and Cowburn, D. (1981) FEBS Lett. 126, 169
53 Seelig, J. (1977) Q. Rev. Biophys. 10, 353