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Proton Nuclear Magnetic Resonance Demonstration of Conformationally Nonequivalent Phospholipid Fatty Acid Chains in Mixed Micelles

Sir:

In the last few years, NMR techniques have been used extensively to examine the structure and packing of phospholipids in multibilayers and sonicated vesicles as membrane models.[1] We have employed these techniques to study phospholipids in mixed micelles[2] which serve as an ideal substrate for lipolytic enzymes such as phospholipase A_2.[3] We have now discovered that, with ^1H NMR, one can detect subtle differences in the environment of the two α-CH_2 groups in the fatty acyl chains of phospholipids, such as dipalmitoylphosphatidylcholine[4] (**1**), in mixed micelles formed with nonionic surfactants such as Triton X-100.[2] Such differences have not been reported for vesicles or multibilayers;[1a-d] mixed micelles with greater motional freedom and narrower line widths[2] provide an ideal membrane-like interface for exploring conformational questions. The differences in the signals from the two chains are not sensitive to changes in chain length or unsaturation of the fatty acids of the phospholipid, but are sensitive to changes in the polar group. Phosphatidylcholine and phosphatidylserine show similar chemical shift differences between the α-CH_2 protons in the *sn*-1 and *sn*-2 fatty acyl chains, whereas, for phosphatidylethanolamine, only a single unresolved peak is observed. This difference in behavior shows that the conformational details or environment of phosphatidylethanolamine is not identical with that of the other two phospholipids.

These findings are of particular interest because the biological significance of these differences can be accessed by the susceptibility of these phospholipids to phospholipase A_2 action. This enzyme specifically catalyzes the hydrolysis of phospholipids by reaction at the carbonyl carbon adjacent to the α-CH_2 group of the *sn*-2 fatty acyl chain.[3b] We[5] have now found that phosphatidylcholine serves as an excellent substrate for phospholipase A_2 in the mixed micelle system, whereas phosphatidylethanolamine is hydrolyzed much more slowly. Phosphatidylserine appears to be a good substrate, but special metal effects complicate the quantitation of its activity.[5] Thus, the activity differences between phosphatidylcholine and phosphatidylethanolamine can be correlated directly with the structural conclusions reported here.

^1H NMR spectra of Triton-phospholipid mixed micelles were obtained at 220 MHz with a Varian HR-220/Nicolet TT-100 pulse Fourier transform system operating at 40 °C. Samples were prepared by adding solutions of Triton X-100 (Rohm and Haas) in D_2O to dry phospholipid; mixing was achieved by a few strokes with a Potter-Elvejhem homogenizer. Dipalmitoylphosphatidylcholine (**1**) and palmitic acid were obtained from Calbiochem. Egg phosphatidylethanolamine, prepared by transesterification of egg phosphatidylcholine, was obtained from Avanti Chemical. Egg phosphatidylcholine was prepared by the method of Singleton et al.,[6] and bovine brain phosphatidylserine was prepared as described elsewhere.[7] Lysophosphatidylcholine was prepared by phospholipase A_2 treatment of egg phosphatidylcholine and purification on alumina chromatography.[3,6] 1-Palmitoyl-2-[2′-$^2H_2$]palmitoylphosphatidylcholine was prepared by the acylation of 1-palmitoyllysophosphatidylcholine with [2′-$^2H_2$]palmitoylimidazolide by a modification of the procedure of Boss et al.[8a] The 1-palmitoyllysophosphatidylcholine was prepared by phospholipase A_2 treatment of dipalmitoylphosphatidylcholine and purification by ether precipitation at pH 3. The [2′-$^2H_2$]palmitoylimidazolide was prepared from [2′-$^2H_2$] palmitic acid (Merck Sharp & Dohme) and carbonyldiimidazole (Aldrich) as described elsewhere.[8b] This acylation procedure results in a small amount of acyl migration between the 1 and 2 positions.[8c] Line widths ($\delta\nu_{1/2}$) were measured as the full width at half-height maximum intensity on expanded spectra; field inhomogeneity was taken to be the line width of the HOD peak and this was subtracted from the reported values.

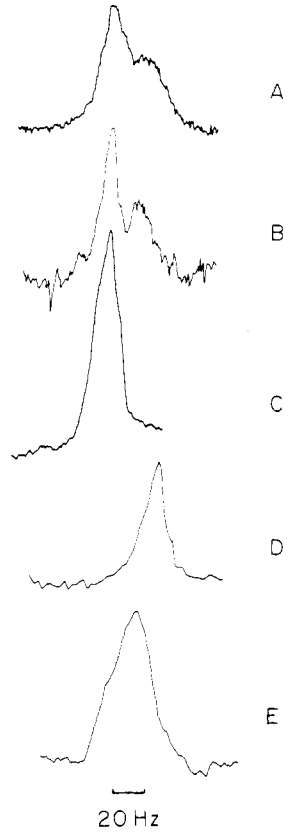
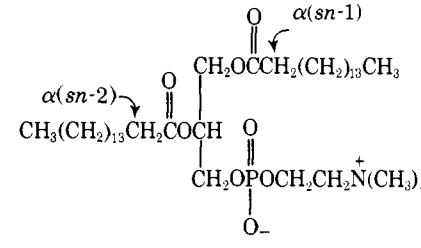


Figure 1. Typical 220-MHz NMR spectra of the α-CH_2 region of various lipids in mixed micelles with Triton X-100 at pH 8.0 and Triton/lipid molar ratios of 4:1: (A) dipalmitoylphosphatidylcholine, (B) same spectrum decoupled by irradiation of the β-CH_2 protons, (C) lysophosphatidylcholine, (D) palmitic acid (molar ratio 8:1), and (E) 1-palmitoyl-2-[2′-$^2H_2$]palmitoylphosphatidylcholine.



1

The α-CH_2 proton signals of phospholipids in mixed micelles are well separated from other resonances in the 220-MHz spectrum.[2a,c] In mixed micelles of Triton and **1**, the α-CH_2 region is composed of two broad overlapping peaks as shown

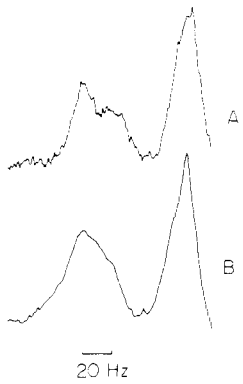


Figure 2. 220-MHz NMR spectra of the α-CH_2 region of (A) phosphatidylserine and (B) phosphatidylethanolamine in mixed micelles with Triton X-100 at pH 6.0 and Triton/phospholipid molar ratios of 4:1. The extra peak furthest upfield arises primarily from the methylene protons adjacent to vinylic groups in the unsaturated fatty acyl chains of the natural phospholipids.

in Figure 1A. Irradiation of the phospholipid β-CH_2 region narrows the α-CH_2 peaks slightly: the narrower decoupled peak is 2.35 ppm downfield from TSP with $\Delta\nu_{1/2}$ = 8–9 Hz, while the broader peak is 20 Hz further upfield (2.26 ppm from TSP) with $\Delta\nu_{1/2}$ = 15–17 Hz (B). The α-CH_2 signals of **1** are compared with those of lysophosphatidylcholine (C) and palmitic acid (D) also in Triton micelles. Each of these products of phospholipase A_2 hydrolysis shows only one resonance in the α-CH_2 region; the line widths ($\Delta\nu_{1/2}$ = 17–18 Hz) correspond to that of the narrow peak in the undecoupled phospholipid spectrum ($\Delta\nu_{1/2}$ = 17–18 Hz).

The nonequivalence of the α-CH_2s may be due to (i) an enhancement of the inherent chemical nonequivalence of the α-CH_2 groups of the two fatty acid chains or (ii) a slow chemical exchange between two distinct micellar environments. The latter could occur if the packing is such that the α-CH_2 group can be in either of two conformations. In this case, the population in each environment (or the exchange rate) should be variable by changing the temperature, pH, surfactant/phospholipid ratio, or phospholipid chain composition. We found that the relative chemical shifts and intensities of the two α-CH_2 resonances in Triton/phosphatidylcholine mixed micelles are not affected by temperature (from 20 to 55 °C),[9] by the surfactant/phospholipid ratio (mole ratios of 2.5:1 to 11:1 Triton:**1**), by changing the phospholipid fatty acid composition (dipalmitoyl- and egg phosphatidylcholine both show the same pattern), or by pH (2 to 9). These experiments suggest that the α-CH_2 pattern exhibited by **1** is an inherent characteristic of the phospholipid conformation in the mixed micelle and not the result of slow exchange between different micelle structures.

The assignment of the two α-CH_2 peaks to the *sn*-1 and *sn*-2 fatty acyl chains was made using a phospholipid in which the *sn*-2 α-CH_2 group was substituted with deuterium as shown in Figure 1E. A single peak was observed at 2.26 ppm. Thus, the broader upfield peak in the spectrum of **1** is assigned to the α-CH_2 group of the *sn*-1 chain, and the narrower downfield peak is assigned to the *sn*-2 chain.

Phosphatidylcholine purified from egg yolk shows the same splitting pattern in the α-CH_2 region when solubilized by Triton X-100 as described for **1**. This suggests that the conformations adapted by the fatty acyl groups are the same for natural phosphatidylcholines containing unsaturated fatty acids as for **1**. Phosphatidylserine in Triton micelles also shows an α-CH_2 region identical with that of **1** as shown in Figure 2A. In both phosphatidylcholine and phosphatidylserine, the peak assigned to the α-CH_2 group of the *sn*-1 chain is shifted upfield from the *sn*-2 chain. An upfield shift could indicate a more hydrophobic environment.[10] The line width of the group is also larger, suggesting more restricted motion or nonequivalence of the two protons of the α-CH_2 group. Surprisingly, phosphatidylethanolamine in Triton does not show the same well-defined α-CH_2 pattern as phosphatidylserine or phosphatidylcholine (Figure 2B). Only one broadened, but somewhat skewed, peak is observed, indicating that the ethanolamine polar group favors a lipid conformation in the mixed micelles in which the environment of the α-CH_2 groups is different from that in phosphatidylcholine or phosphatidylserine.

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Nature of Hg^{2+}–L-Cysteine Complexes Implicated in Mercury Biochemistry

Sir:

L-Cysteine complexes of Hg^{2+} are urinary excretory products of certain mercurial diuretics[1] and have been directly implicated in the transport of Hg^{2+} across membranes,[2] the binding of Hg^{2+} to kidney proteins,[3] and the role of metallothioneine as a possible detoxificating agent for low levels of inorganic mercury.[4] Despite this, little is known of the structures of Hg^{2+}–L-cysteine complexes and as a recent review indicates[5] even the formulae and stoichiometries of the simplest compounds are controversial. We have carried out detailed synthetic, spectroscopic, and x-ray structural studies of Hg^{2+}–L-cysteine complexes to establish structural details of