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Headgroup interactions in mixed phospholipid bilayers

(deuterium NMR/membrane surface structure/binary lipid bilayers)

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ABSTRACT **^{2}H NMR methods have been used to study how bilayer-forming phospholipids interact with each other at the membrane surface. Aqueous dispersions of dimyristoyl-*sn*-phosphatidylcholine (Myr_2-PtdCho), dimyristoyl-*sn*-phosphatidylethanolamine (Myr_2-PtdEtn), and dimyristoyl-*sn*-phosphatidyl-3-glycerol, specifically deuterated at different positions in their headgroups, give well-resolved ^{2}H NMR powder spectra. These spectra are characteristic of a lipid bilayer with quadrupole splittings that are sensitive to the amplitude of headgroup motion. In binary mixed bilayers of deuterated lipids with an unlabeled component, all parts of the deuterated headgroup monitor the presence of the second lipid from changes in the measured quadrupole splittings. The headgroups of the charged lipids, dimyristoyl-*sn*-phosphatidylserine and dimyristoyl-*sn*-phosphatidyl-3-glycerol, interact to the largest extent with the choline moiety of Myr_2-PtdCho and the ethanolamine moiety of Myr_2-PtdEtn, whereas a somewhat smaller but still marked alteration in headgroup motion was observed in Myr_2-PtdCho/Myr_2-PtdEtn mixtures. The large changes in the deuterium quadrupole splittings for the zwitterionic lipids after addition of a charged lipid suggest that either a strong perturbation in the hydrogen bonding occurs or changes take place in the water structure at the membrane surface, or possibly both.**

The lipid component of biological membranes is usually composed of a limited number of phospholipid types, which are identified by the chemical nature of their polar headgroup. Both the conformational and motional properties of lipid headgroups are well characterized for model membranes containing single phospholipid species, mainly on the basis of proton, deuterium, and phosphorus NMR and neutron diffraction studies (1–5). In particular, ^{2}H NMR has been used to investigate conformational changes induced by ions, cholesterol, and anesthetics (6–8). However, limited information is available on lipid structure at membrane surfaces if more than one naturally occurring phospholipid type is present (9–12).

Here we present results from ^{2}H NMR studies of fully hydrated bilayers of phospholipids deuterated in their headgroups, which suggest that strong interactions occur at the membrane surface between different lipids in binary mixed bilayers. In particular, phospholipids that carry a residual negative charge significantly change the measured quadrupole splittings for zwitterionic lipids. These observations imply that headgroup reorientations and motional amplitude changes can be induced in mixed lipid bilayers by alterations in the structured water layer at the membrane surface.

MATERIALS AND METHODS

Materials. The following specifically deuterated dimyristoyl derivatives of *sn*-phosphatidylcholine (Myr_2-PtdCho), phosphatidyl-3-glycerol (Myr_2-PtdGro), and phosphatidylethanolamine (Myr_2-PtdEtn) were synthesized as described (9): R—O—$\overset{\alpha}{C^2H_2}$—$\overset{\beta}{C^2H_2}$—$\overset{\gamma}{N^+}(CH_3)_3$ = Myr_2-PtdCho-d_4; R—O—CH_2—CH_2—$N^+(C^2H_3)_3$ = Myr_2-PtdCho-d_9; R—O—C^2H_2—C^2H_2—N^+H_3 = Myr_2-PtdEtn-d_4; and R—O—C^2H_2—C^2HOH—CH_2OH = Myr_2-PtdGro-d_3.

Nondeuterated dimyristol-*sn*-phosphatidylserine (Myr_2-PtdSer) was prepared by phosphorylation of dimyristoylglycerol with phosphorus oxychloride, followed by esterification with *N*-carbobenzoxyserine benzyl ester by standard procedures (13). The protecting groups were removed in formic acid/tetrahydrofuran by using a palladium-impregnated polymer (Pierce) as catalyst.

Sample Preparation. Samples were prepared from stock solutions of each lipid in chloroform/methanol, 2:1 (vol/vol), by mixing suitable amounts to yield the desired composition. Solvent was removed first with nitrogen and then under high vacuum overnight. Hydration of membranes was performed by addition of 0.1 M Tris·HCl (pH 7.5) and mixing for 5 min above the lipid gel/liquid crystalline phase-transition temperature.

^{2}H NMR Measurements. ^{2}H NMR spectra were accumulated on a Bruker WH 300 spectrometer with single 90° pulses of 29-μsec duration at 46.1 MHz. Temperatures were controlled to ±2°C by using a nitrogen gas flow.

RESULTS AND DISCUSSION

The quadrupole splittings, $\Delta\nu_Q$, for the headgroup segments of specifically deuterated Myr_2-PtdCho, Myr_2-PtdEtn, and Myr_2-PtdGro in fully hydrated bilayers were measured by ^{2}H NMR in binary mixed bilayers with unlabeled Myr_2-PtdGro, Myr_2-PtdSer, or Myr_2-PtdEtn. Figs. 1 and 2 show typical ^{2}H NMR spectra for one of these binary mixtures containing Myr_2-PtdCho (both -d_4 and -d_9 separately) in mixtures with Myr_2-PtdSer. All three headgroup segments of Myr_2-PtdCho were found to be sensitive to the presence of Myr_2PtdSer. Values of $\Delta\nu_Q$ for the $N^+(C^2H_3)_3$ and the β-C^2H_2 groups decreased with increasing Myr_2-PtdSer concentration (Fig. 3), whereas an increase of $\Delta\nu_Q$ was observed for the α-C^2H_2 segment. Also, the splitting between the two α-C^2H_2 NMR lines for Myr_2-PtdCho-d_4 was larger in mixed Myr_2-PtdCho/Myr_2-PtdSer bilayers than in pure Myr_2-PtdCho.

Mixed bilayers containing deuterated Myr_2-PtdCho and Myr_2-PtdGro gave similar but even more substantial changes in the quadrupole splittings compared to those from Myr_2-PtdCho/Myr_2-PtdSer dispersions (Table 1). Unlabeled Myr_2-PtdGro caused an increase in the α-C^2H_2 quadrupole splitting of Myr_2-PtdCho-d_4 and a decrease for both the β-C^2H_2 and the $N^+(C^2H_3)_3$ segments. The quadrupole splittings for the β-C^2H_2 and the

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Abbreviations: Myr_2-PtdCho, dimyristoyl-*sn*-phosphatidylcholine; Myr_2-PtdGro, dimyristoyl-*sn*-phosphatidyl-3-glycerol; Myr_2-PtdEtn, dimyristoyl-*sn*-phosphatidylethanolamine; Myr_2-PtdSer, dimyristoyl-*sn*-phosphatidylserine.

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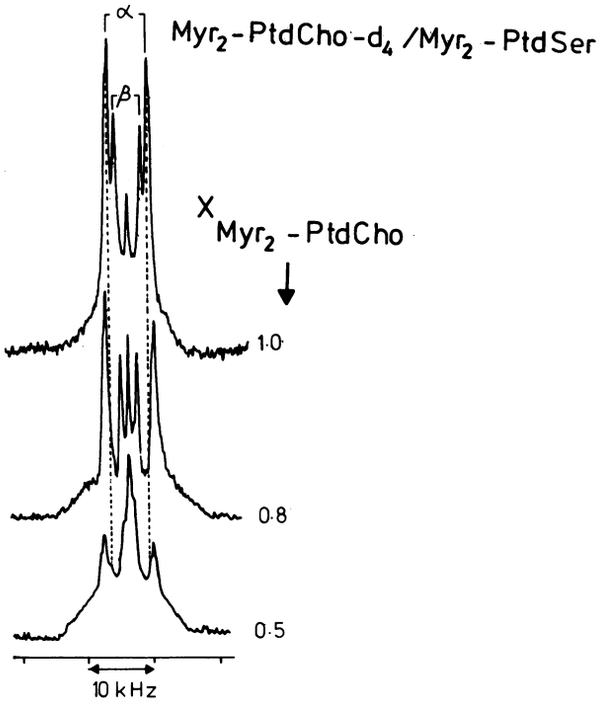

FIG. 1. ^2H NMR spectra (46.1 MHz) of mixed Myr_2-PtdCho-d_4/Myr_2-PtdSer (Myr_2-PtdCho deuterated at the α- and β-methylenes of the choline headgroup) bilayers at 50°C. Samples contained 60–80 mg of deuterated Myr_2-PtdCho. Spectra were recorded by using single 90° pulses. The spectral width was 50 kHz, and 2,000–6,000 free induction decays were accumulated.

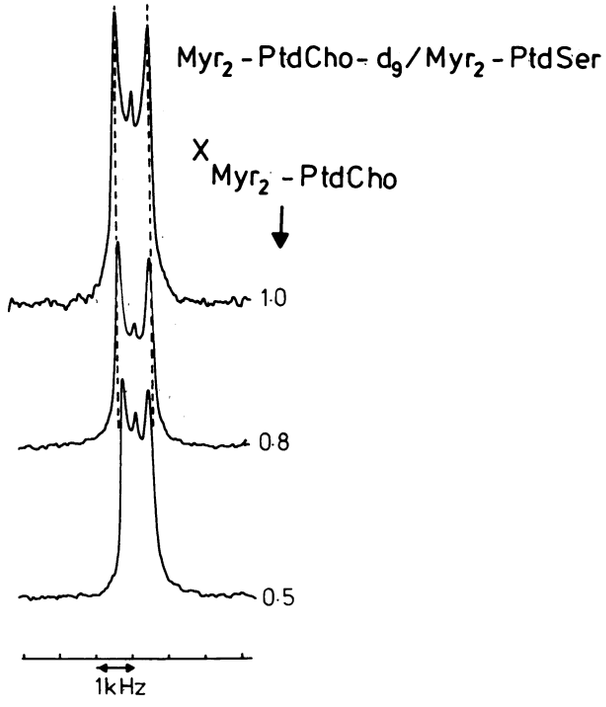

FIG. 2. ^2H NMR spectra (46.1 MHz) of Myr_2-PtdCho-d_9/Myr_2-PtdSer mixtures [Myr_2-PtdCho deuterated at the $N^+(C^2H_3)_3$ position on the headgroup] at 50°C. Conditions are as in Fig. 1, and samples contained about 10 mg of Myr_2-PtdCho-d_9; 500 free-induction decays were recorded with a spectral width of 20 kHz.

$N^+(C^2H_3)_3$ groups almost collapsed when the molar fraction, $X_{Myr_2\text{-}PtdCho}$, approached zero in Myr_2-PtdCho/Myr_2-PtdGro mixtures.

These indications of charged lipid perturbation of the PtdCho headgroups can be rationalized in at least one or both of two possible ways. First, the presence of either Myr_2-PtdSer or Myr_2-PtdGro only changes the torsion angles in the choline moiety but leaves the amplitude of motion in the headgroup of Myr_2-PtdCho more or less unaffected. If the average orientation of the C—^2H bonds in the β-methylene and the $N^+(C^2H_3)_3$ positions comes closer to the "magic angle" of 55° with respect to the bilayer normal, a decrease of the particular $\Delta\nu_Q$ values would be observed. The change of the α-C^2H_2 quadrupole splitting in this case may be caused by quite a small conformational reorientation (6), which is confirmed by the insensitivity of the ^{31}P NMR chemical shift anisotropy to lipid headgroup interactions (9). Spin lattice relaxation times for all three headgroup segments of Myr_2-PtdCho are very similar in pure and in mixed Myr_2-PtdCho/Myr_2-PtdGro bilayers (9), supporting the assumption of a mainly conformational change to account for the quadrupole splitting changes reported here. Second, the smaller steric sizes of the Myr_2-PtdSer and Myr_2-PtdGro headgroups compared with that of the choline residue also suggests that the amplitude of the choline headgroup motion may be altered. In the presence of the small serine or glycerol group, the bulky Myr_2-PtdCho headgroup can sweep more of the bilayer surface such that the rotational freedom around the —C(α)—C(β) axis may be increased, resulting in the observation of much smaller $\Delta\nu_Q$ values for the β-C^2H_2 and the $N^+(C^2H_3)_3$ groups.

In contrast to the experiments with mixed bilayers containing deuterated Myr_2-PtdCho, the motion of the smaller charged headgroup of deuterated Myr_2-PtdGro seems to be restricted on mixing into unlabeled Myr_2-PtdCho bilayers, as indicated by the larger quadrupole splittings of the α-C^2H_2 and the β-C^2H segment of Myr_2-PtdGro-d_3 (Table 1). The perturbation of one lipid headgroup on the other in this case either may arise from *direct* molecular interactions or be mediated through hydrogen bonding or structural changes in the water layer at the membrane surface or both. If only direct molecular contacts between the headgroups take place, one would expect results for PtdCho/

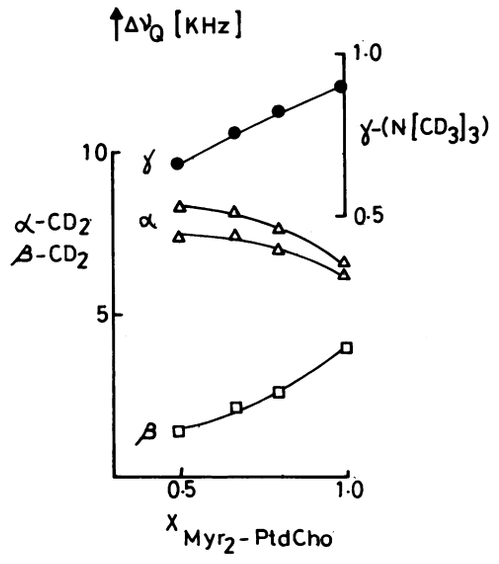

FIG. 3. Deuterium quadrupole splittings, $\Delta\nu_Q$, of the α- and β-methylene and the $N^+(C^2H_3)_3$ groups of headgroup-deuterated Myr_2-PtdCho in mixtures with Myr_2-PtdSer as a function of molar fraction $X_{Myr_2\text{-}PtdCho}$. Conditions are the same as in Figs. 1 and 2.

Table 1. Relative percentage changes of quadrupole splittings for headgroup-deuterated Myr_2-PtdCho, Myr_2-PtdEtn, and Myr_2-PtdGro*

Label position in headgroup	Relative changes of $\Delta\nu_Q$, %: Myr_2-PtdCho-($-d_4$ and $-d_9$) with Myr_2-PtdSer	Myr_2-PtdGro	Myr_2-PtdEtn	Myr_2-PtdEtn-$-d_4$ with Myr_2-PtdCho	Myr_2-PtdGro	Myr_2-PtdGro-$-d_3$ with Myr_2-PtdCho	Myr_2-PtdEtn
α-C^2H_2	+20	+48	−12	+8	+22	+3	+3
β-$C^2H_{(2)}$	−65	−75	−13	0	−60	+130	+65
γ-N^+-$(C^2H_3)_3$	−25	−45	−10				

*After the addition of an equimolar amount of nondeuterated Myr_2-PtdSer, Myr_2-PtdGro, Myr_2-PtdEtn, and Myr_2-PtdCho. All values are for a temperature 20 ± 2°C above the main phase transition of the particular mixture. Negative and positive values refer to a decrease and increase, respectively, in quadrupole splitting when compared with the pure labeled lipid.

PtdEtn mixtures similar to those for the PtdCho/PtdSer and PtdCho/PtdGro bilayers because the ethanolamine headgroup is even smaller than the serine and glycerol residues. Table 1 shows that significant changes in the quadrupole splittings of all Myr_2-PtdCho headgroup segments and of the α-C^2H_2 position of deuterated Myr_2-PtdEtn are indeed observed in mixed bilayers. However, the $\Delta\nu_Q$ values in equimolar Myr_2-PtdCho/Myr_2-PtdEtn membranes do not differ by more than 15% from those measured for the pure lipids. This suggests that hardly any significant reorientation is induced in either the Myr_2-PtdCho or Myr_2-PtdEtn headgroups by the presence of the other lipid. On the other hand, changes of quadrupole splittings for Myr_2-PtdEtn-d_4 after addition of Myr_2-PtdGro are similar to those observed for deuterated Myr_2-PtdCho (Table 1). It is known from nuclear Overhauser experiments (14) and x-ray studies (15) that the $N^+(CH_3)_3$ group of PtdCho and the N^+H_3 group of PtdEtn can interact with the phosphates of neighboring lipid molecules. In pure PtdEtn bilayers, this interaction is stabilized by hydrogen bonding (15). Thus, the introduction of a negatively charged lipid component into Myr_2-PtdCho or Myr_2-PtdEtn membranes may alter the hydrogen bonding or modify the water layer, or both, resulting in conformational changes of both the charged and the neutral headgroups.

The most surprising result of our experiments is the large degree to which lipid headgroups at the membrane surface may be perturbed by the presence of another lipid type. Even if two phospholipid species, like Myr_2-PtdCho and deprotonated Myr_2-PtdGro, have similar molecular structures and identical thermodynamic properties (9), variations of $\Delta\nu_Q$ values are obtained that exceed the effect of "phospholipid-unlike" compounds, such as cholesterol (7) or the local anesthetic tetracaine hydrochloride (8). Consequently it is clear that ^2H NMR studies of lipid bilayers that contain, for example, one of the latter compounds or a reconstituted membrane protein must be interpreted with caution. Straightforward conclusions about lipid headgroup properties in biological membranes may, in such membrane systems, be complicated and masked by the effects caused by different phospholipid types interacting with each other. On the other hand, the sensitivity of the approach could, when sufficient controls have been carried out, be potentially very useful for studying membrane properties at the apolar/polar membrane interface.

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