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Dynamic Structure and Phase Behavior of Dimyristoylphosphatidylethanolamine Bilayers Studied by Deuterium Nuclear Magnetic Resonance†

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ABSTRACT: The dynamic structure of dimyristoylphosphatidylethanolamine bilayers has been studied by deuterium nuclear magnetic resonance spectroscopy of the perdeuterated *sn*-2 chain. The order parameter profile of the lipid chains in the fluid phase is qualitatively similar to that found for other phospholipids, but the order parameter plateau is ca. 15% higher than found for dimyristoylphosphatidylcholine at a comparable reduced temperature. The chains of dimyristoylphosphatidylethanolamine undergo a segmental motion in the gel phase, which for segments close to the end of the chain approximates continuous axial diffusion. In the phase-transition region, spectra are observed that can be best described in terms of the interconversion of coexisting lipid phases through the transition.

The lipid composition of biological membranes shows diversity both in the hydrocarbon-chain content and in the different phospholipid head-group classes [see, e.g., Marsh (1975)]. The hydrocarbon-chain composition is normally regulated such that the lipids are in a fluid, liquid-crystalline state at physiological temperatures (Chapman, 1975). The functional implications of the lipid head-group diversity are less clear. One effect is presumably to modulate the surface properties of the membrane, especially in the case of negatively charged lipid head groups. Another possibility is that the degree of fluidity and chain ordering may vary between the different lipid classes, and this could provide a mechanism whereby the dynamic properties of a fluid membrane could be finely adjusted by varying the phospholipid head-group composition. If this mechanism were operative, spatially differentiated areas of varying fluidity might also be created by fluid–fluid phase separation between different lipid classes. This would provide a method of regulating membrane dynamics in a progressive manner, without the disruptive effects associated with the gel–fluid bilayer phase transition or the pronounced ordering effects produced by cholesterol.

Two of the principal lipid classes in mammalian membranes are the zwitterionic lipids phosphatidylcholine and phosphatidylethanolamine. In addition, phosphatidylethanolamine is the major membrane phospholipid in many microorganisms. In the present paper, we have compared the chain ordering in phosphatidylethanolamines with that in the much more studied phosphatidylcholines, using deuterium nuclear magnetic resonance (NMR).[1] It is found, by comparing the dimyristoyl derivatives with a perdeuterated *sn*-2 chain, that the chain ordering in phosphatidylethanolamine is significantly greater than in phosphatidylcholine at the same reduced temperatures. In addition, we have investigated the dynamic properties of the dimyristoylphosphatidylethanolamine chains both in the gel phase and through the gel-to-fluid phase transition.

Materials and Methods

Perdeuterated myristic acid was obtained from Merck Sharp & Dohme, Canada Ltd. 1-Myristoyllysophosphatidylcholine (Calbiochem, Giessen, West Germany) was acylated with the carboxylimidazole complex of perdeuterated myristic acid

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[1] Abbreviations: DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance.

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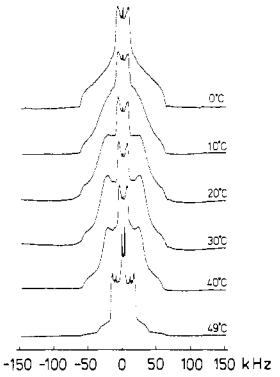

FIGURE 1: Temperature dependence of 46.1-MHz ^2H NMR spectra of *sn*-2 perdeuterated dimyristoylphosphatidylethanolamine bilayers.

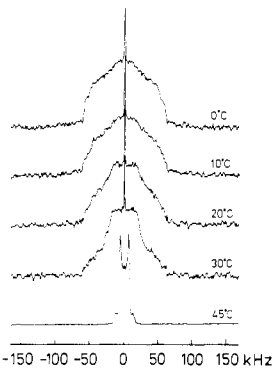

FIGURE 2: Temperature dependence of 46.1-MHz ^2H NMR spectra of *sn*-2 [13,13-$^2H_2$]dimyristoylphosphatidylethanolamine bilayers.

according to the method of Boss et al. (1975). Dimyristoylphosphatidylcholine with perdeuterated myristic acid as the *sn*-2 chain was converted to the corresponding phosphatidylethanolamine by base exchange catalyzed by phospholipase D (Boehringer, Mannheim, West Germany) according to the method of Comfurius & Zwaal (1977). [13,13-$^2H_2$]Myristic acid was prepared by reduction of 13-ketomyristic acid methyl ester (Hubbell & McConnell, 1971) in two stages with $NaBD_4$ and $LiAlD_4$. The [13,13-$^2H_2$]myristyl alcohol was oxidized to myristic acid with CrO_3 (Tulloch, 1979). The [13,13-$^2H_2$]myristic acid was used to prepare 1,2-dimyristoyl-*sn*-glycerol (Eibl, 1980), which was then phosphorylated to produce 1,2-[13,13-$^2H_2$]dimyristoyl-*sn*-glycero-3-phosphoethanolamine (Eibl, 1978).

Phospholipids were hydrated in excess bidistilled normal water (65–75% w/w) by heating to 60 °C with thorough mixing. ^2H NMR spectra were recorded on a Bruker CXP-300 spectrometer at 46.1 MHz, using the quadrupolar echo technique (Davis et al., 1976). Pulse widths were 2.5 or 5 μs; interpulse spacing was 40–50 μs; recycle time was 0.5 or 1.0 s; sweep width was 125 or 500 kHz. Approximately 2000–3000 scans were recorded for the gel-phase spectra and approximately 600 scans for the fluid-phase spectra if recorded separately.

Results

The 46.1-MHz ^2H NMR spectra of dimyristoylphosphatidylethanolamine perdeuterated in the *sn*-2 chain are given in Figure 1. In these spectra, data collection conditions were optimized for the gel phase. The spectra extend out to a maximum range of 120 kHz and are temperature dependent, indicating the presence of (slow) molecular motion in the gel phase. The change to a much narrower, sharper spectrum occurs at the gel–fluid phase transition at ~48 °C. The ^2H NMR spectra of dimyristoylphosphatidylethanolamine specifically deuterated at the 13-position of the fatty acid chains are given in Figure 2. Again, the spectra in the gel phase are temperature dependent and extend out to a maximum spectral range of 120 kHz, with conversion to a narrower spectrum at the gel–fluid transition at ~45 °C.[2] The exact nature of the gel-phase spectra depends on sample history; the spectra in Figure 2 were from a sample that was preincubated at 4 °C for 2–3 days. Samples of [13,13-$^2H_2$]DMPE that had not been incubated at low temperature showed a greater degree of motional averaging (data not shown). The data given in Figure 1 correspond to a sample that had not been incubated.

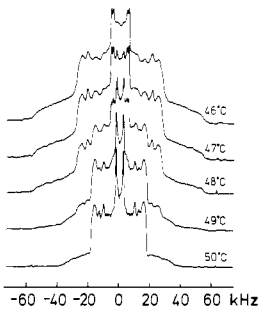

FIGURE 3: ^2H NMR spectra of *sn*-2 perdeuterated dimyristoylphosphatidylethanolamine through the gel–fluid phase transition.

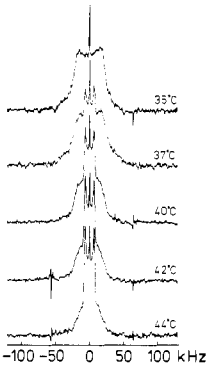

FIGURE 4: ^2H NMR spectra of *sn*-2 [13,13-$^2H_2$]dimyristoylphosphatidylethanolamine through the gel–fluid phase transition.

^2H NMR spectra of DMPE perdeuterated in the *sn*-2 chain, at various temperatures throughout the main gel–fluid phase transition, are given in Figure 3. The spectra are all apparently two component, converting from the broad powder pattern observed in the gel phase immediately below the transition to the narrower, sharper powder pattern observed in the fluid phase immediately above the transition. Pairwise subtractions between the spectra of Figure 3 yield end points that approximate, but do not correspond exactly, to single-component powder patterns. The lack of exact correspondence may be because the spectra due to the two component states are temperature dependent, or alternatively, there may be exchange between the components, possibly modulated by echo distortions (Rice et al., 1981). The ^2H NMR spectra of DMPE specifically deuterated at the 13-position at various temperatures through the phase transition are given in Figure 4. Again, the spectra apparently consist of two components with progressively changing relative proportions.

Typical ^2H NMR spectra of DMPE perdeuterated in the *sn*-2 chain, in the fluid phase, are given in Figures 1 and 3. A large number of different peaks are resolved corresponding to the different deuterated $-CD_2$ segments of the DMPE *sn*-2 chain. The measured quadrupole splittings are compared with

[2] The sharp, central lines in the spectra of Figures 2 and 4 arise from deuterium at natural abundance in the water used to hydrate the lipid.

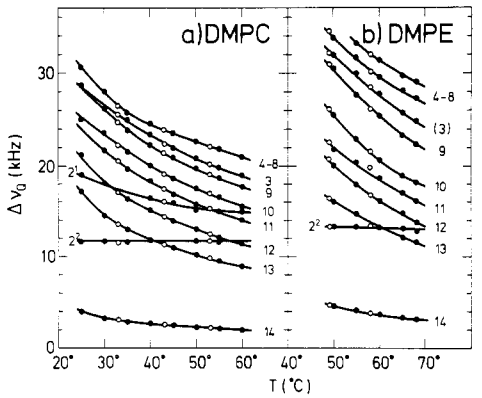

FIGURE 5: Temperature dependence of quadrupole splittings in the ^{2}H NMR spectra of (a) *sn*-2-chain perdeuterated dimyristoylphosphatidylcholine and (b) *sn*-2 perdeuterated dimyristoylphosphatidylethanolamine. Filled symbols represent increasing temperature, and open symbols represent decreasing temperature.

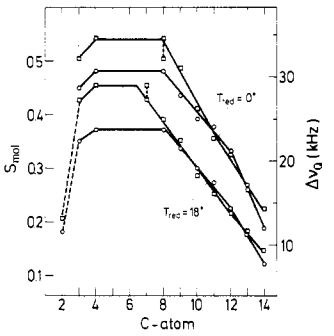

FIGURE 6: Order-parameter profiles of *sn*-2 chain in dimyristoylphosphatidylcholine (○) and dimyristoylphosphatidylethanolamine (□) bilayers at T_{red} = 0 and 18 °C, deduced from the quadrupole splittings of Figure 5. The C-2 data refer only to the right-hand ordinate (quadrupole splitting), and the C-14 data refer only to the left-hand ordinate (order parameter).

those from DMPC perdeuterated in the *sn*-2 chain, in Figure 5. The resonances for DMPC in Figure 5 have been assigned in comparison with published values for specifically deuterated DMPC's (Oldfield et al., 1978). The resonances from positions 10 to 14 of DMPE in Figure 5 have been assigned by comparison with DMPC, as is also that from C-2^2, one of the two inequivalent deuterons at position 2. The resonance from C-2^1, the other deuteron at position 2, is not resolved, because of overlap and its intrinsically low intensity. Assignment of the resonance due to position 13 was also confirmed by comparison with the specifically deuterated DMPE. The resonances for positions 3–9, which lie on the order-parameter "plateau", all have a larger quadrupole splitting than the corresponding DMPC resonances at the same reduced temperature. In addition, four peaks are resolved in this region for DMPE, compared with three peaks for DMPC. For this reason, the assignment of the resonances for positions 3–8 of DMPE is somewhat tentative, based on the temperature dependence and anticipated order-parameter profile. The order-parameter profiles of DMPC and DMPE are compared at reduced temperatures[3] of 0 and 18 °C in Figure 6. It is clear that the quadrupole splittings are significantly greater for DMPE than for DMPC for one of the deuterons at position 2, for position 14, and for positions 3–8 in the plateau region. In the intermediate region C-10 to C-13, where the order parameter changes most steeply with position, the quadrupole splittings are approximately the same.

Discussion

Gel Phase. Both the maximum extent of the quadrupole splitting of the methylene groups and the perpendicular quadrupole splitting of the methyl group, which have values of 120 and 16 kHz, respectively, in Figure 1, are much smaller than the corresponding values of 250 and 42 kHz predicted for completely rigid chains. This demonstrates the existence of chain motion and disorder in the gel phase of DMPE, as has previously been found for phosphatidylcholine (Davis, 1979) and galactosylcerebroside (Huang et al., 1980) by ^{2}H NMR and for phosphatidylcholines and phosphatidylglycerols by saturation transfer electron spin resonance (Marsh, 1980; Watts & Marsh, 1981). The quadrupole splitting of the terminal methyl group varies from $\Delta\nu_Q^{\perp}$ = 16 kHz at 0 °C to 12 kHz at 40 °C in the gel phase. This is considerably less than the value of $\Delta\nu_Q^{\perp}$ = 21 kHz that would be obtained for a freely rotating methyl group in an all-trans chain that is rotating rapidly about its long axis. The smaller quadrupole splitting for the terminal methyl group immediately suggests that there is segmental motion of the lipid chains in the gel phase, independent of whether the chains are rotating about their long axes. The spectra of the methylene groups are difficult to interpret because of overlapping resonances from the perdeuterated chain. Recently, a detailed analysis of the ^{2}H NMR spectra of specifically deuterated dipalmitoylphosphatidylethanolamines has been presented by Blume et al. (1982). The spectra in the gel phase were interpreted in terms of axial diffusion with limited trans–gauche isomerism. The present results on the methylene resonances are consistent with this interpretation. At higher temperatures, there is a concentration of intensity in peaks with a splitting of ca. 50 kHz (Figure 1), which is less than the value of 63 kHz expected for rotating all-trans chains, indicating the presence of limited rotational isomerism.

For the specifically deuterated sample incubated at 4 °C, the low-temperature spectra in Figure 2 are similar to those simulated by Blume et al. (1982), assuming an intermediate axial rotation rate $\sim 3 \times 10^5$ s^{-1} and very limited trans-gauche isomerism. At higher temperatures (see 35 °C, Figure 4), an axial spectrum with a splitting of ca. 32 kHz is obtained. This is considerably less than both the 63-kHz splitting expected for a rigidly rotating chain and the 50-kHz maxima observed with perdeuterated DMPE at the same temperature. Axial rotation of the all-trans chain or dynamic tilting of the whole molecule cannot account for these differential effects. Thus there is clear evidence for the existence of segmental motion of the DMPE chains at the higher temperatures in the gel phase. For galactosylcerebroside, it has been demonstrated that segmental motion takes place with*out* axial diffusion (Huang et al., 1980), whereas the results of Blume et al. (1982) clearly demonstrate that axial diffusion is additionally present in the phosphatidylethanolamine gel phase.

A recent X-ray diffraction study (Seddon et al., 1983) has demonstrated the metastability and polymorphic behavior of dilaurylphosphatidylethanolamine bilayers in the gel phase. Upon incubation at low temperature, the gel phase transforms to a less hydrated, more crystalline phase. The present results on incubated samples (Figure 2) demonstrate the corresponding dynamic changes taking place in the low-temperature

[3] Reduced temperature is defined here as the corresponding temperatures above the phase transition: $T_{red} = T - T_t$, where T_t is the gel–fluid transition temperature. An alternative definition, $\theta = (T - T_t)/T_t$, has been used elsewhere (Seelig & Browning, 1978), but the transition temperatures of DMPC and DMPE are sufficiently close on the absolute scale that differences between the two definitions can be neglected here. For T_{red} = 18 °C, the difference would correspond to a 1.5 °C shift in temperature, which does not materially affect the conclusions. For T_{red} = 0, the two definitions are, of course, equivalent.

phase of dimyristoylphosphatidylethanolamine.

Phase Transition. The spectra of Figure 3 apparently correspond to the interconversion of a powder pattern of broader splitting to a coexisting one of narrower splitting, on passing through the phase transition at 48 °C. Since the broader splittings bear no simple relation to the narrower splittings and both subspectra resemble those at the limits of the gel–fluid transition, and since similar effects are also observed with phosphatidylethanolamine spin-labels (J.-H. Sachse, A. Watts, and D. Marsh, unpublished results), which are not subject to quadrupolar echo distortions, it seems clear that the spectral effects correspond to a coexistence of the two different lipid phases during the gel-to-fluid phase transition. The phase transition of phosphatidylethanolamines is known to be considerably broader than for phosphatidylcholines and is also asymmetrically broadened toward lower temperatures (Mabrey & Sturtevant, 1978). It is thus not surprising that the phase transition consists of the interconversion of gel and fluid lipid chains [cf. Marsh et al. (1976, 1977)]. It is interesting to note that coexistence of gel and fluid phases has previously been demonstrated in membranes of *Acholeplasma laidlawii* (Smith et al., 1979; Smith, 1981). The spectra of DMPE specifically labeled at the 13-CD_2 group show similar effects of coexistence, although occurring at somewhat lower temperatures. The apparent temperature shift most probably arises partly from the high sensitivity of these spectra to the presence of the fluid phase and also perhaps because the asymmetry of the transition in some way involves segments close to the end of the lipid chains.

Fluid Phase. Independent of the uncertainties in the assignment of some of the lines in the spectra of perdeuterated DMPE, it is clear from Figure 6 that the plateau section of the order parameter, from C-3 to C-8, is distinctly higher in DMPE than in DMPC. This demonstrates a quantitative difference in the dynamic behavior of DMPE bilayers from those of DMPC, even when the comparison is made on a reduced temperature scale. The result is also independent of any ambiguity that might arise as a result of the line overlap in the spectra of perdeuterated chains, since the comparison is made between perdeuterated chains in both cases. This increased order parameter in phosphatidylethanolamine bilayers correlates rather well with the smaller surface area per molecule relative to phosphatidylcholine bilayers, which is observed by X-ray diffraction (J. M. Seddon, G. Cevc, R. Kaye, and D. Marsh, unpublished results). The closer molecular packing corresponds to the decreased amplitude of chain motion in the phosphatidylethanolamine bilayers.

In addition to the differences in the plateau region, the order parameter of the terminal methyl group is significantly larger in DMPE than in DMPC, as also is that of the C-2^2 position. Nonetheless, in the latter case, the special features of the bent *sn*-2 chain (Seelig & Browning, 1978) are still present. Thus, although the qualitative features of the order-parameter profile are preserved over a wide range of different phospholipids (Seelig & Browning, 1978), there are distinct quantitative differences between the degree of ordering of the lipid chains in phosphatidylcholines and phosphatidylethanolamines. The difference between the two in the plateau region is equivalent to the effect of an approximately 20 °C increase in temperature for DMPC or adding 15–20 mol % cholesterol to DMPC above the phase transition [cf. Jacobs & Oldfield (1979)]. Therefore, it does appear that the dynamic structure of cell membranes could be modulated by changes in phospholipid classes.

Acknowledgments

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Registry No. DMPE, 998-07-2.

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