Comparison of phosphatidylethanolamines and phosphatidylcholines

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ABSTRACT The role of lipid diversity in biomembranes is one of the major unsolved problems in biochemistry. One parameter of possible importance is the mean cross-sectional area occupied per lipid molecule, which may be related to formation of nonbilayer structures and membrane protein function. We have used $^2$H NMR spectroscopy to compare the properties of 1,2-diperdeuteriopalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-d$_{62}$) and 1,2-diperdeuteriopalmitoyl-sn-glycero-3-phosphocholine (DPPC-d$_{62}$) in the $L_a$ phase. We find that DPPE has greater segmental order than DPPC, and that this increase in order is related to the smaller area per acyl chain found for DPPE. Values of the mean cross-sectional chain area are calculated using a simple diamond lattice model for the acyl chain configurational statistics, together with dilatometry data. The results obtained for the mean area per molecule are comparable with those from low angle x-ray diffraction studies.

INTRODUCTION

The significance of lipid diversity in biological membranes still remains obscure. A greater understanding of the differences between the principal lipid constituents in eukaryotic systems, such as phosphatidylcholine and phosphatidylethanolamine, could help resolve this problem. One excellent technique to study phospholipids is deuterium nuclear magnetic resonance (NMR) spectroscopy, which has been used widely to study the average configurational properties and molecular dynamics of phospholipid systems (Seelig, 1977; Seelig and Seelig, 1980; Davis, 1983; Salmon et al., 1987; Dodd and Brown, 1989; Brown et al., 1990; Brown and Söderman, 1990). The bulk of the work has been directed toward phosphatidylethanolamines; however, a few groups have applied $^2$H NMR spectroscopy to investigate phosphatidylethanolamines. Seelig and Gally (1976) studied the polar head group of 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) using $^2$H NMR and obtained evidence suggesting that the phosphoethanolamine moiety undergoes rapid transitions between two enantiomeric conformations, each of which lies approximately flat on the surface of the bilayer. Blume et al. (1982a,b), in a series of papers, showed for DPPE deuterated specifically in three acyl positions that the chains are approximately all-trans in the low temperature ordered state with the molecules rotating about their long axes. Finally, Marsh et al. (1983) studied the phase behavior and configurational order of sn-2 chain perdeuterated 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-d$_{62}$) in the $L_a$ phase, concluding that the order parameter plateau of the phosphatidylethanolamine was higher compared with the corresponding phosphatidylcholine (DMPC-d$_{62}$).

$^2$H NMR spectroscopy has also been used to estimate the effective acyl chain lengths and average chain cross-sectional areas of phospholipids in the liquid-crystalline state in terms of simple statistical models (Seelig and Seelig, 1974; Schindler and Seelig, 1975; Salmon et al., 1987; DeYoung and Dill, 1988; Ipsen et al., 1990). The cross-sectional chain area is related to the mean area occupied by a lipid molecule at the membrane lipid-water interface. The area per molecule in turn is important because the effective or average shape of the molecules is associated with the geometry of lipid aggregates (Israelachvili et al., 1976; Tanford, 1980). The latter reflect various contributions to the free energies of lipid systems which may play a role in regulating membrane protein function (Kirk et al., 1984; Wieslander et al., 1986; Wiedmann et al., 1988). In this

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$^1$Abbreviations used in this paper: DLPE, 1,2-dilauryl-sn-glycero-3-phosphoethanolamine; DMPC-d$_{62}$, 1-myristoyl-2-perdeuteriomyristoyl-sn-glycero-3-phosphoethanolamine; DMPE, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine; DMPE-d$_{62}$, 1-myristoyl-2-perdeuteriomyristoyl-sn-glycero-3-phosphoethanolamine; DPPC, 1,2-dipalmi­toyl-sn-glycero-3-phosphocholine; DPPC-d$_{62}$, 1,2-diperdeuteriopalmitoyl-sn-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; EDTA, ethylenediaminetetraacetic acid; egg PE, egg yolk phosphatidylethanolamine; $^2$H NMR, deuterium nuclear magnetic resonance; $T_{m_p}$, inverted hexagonal phase; $T_m$, liquid-crystalline phase; MOPS, morpholinopropane sulfonic acid; $S_{ij}$, carbon-deuterium bond order parameter of $i$th segment; TLC, thin layer chromatography; $T_{red}$, main (gel to liquid-crystalline) phase transition temperature; $T_{red}$, reduced temperature.
paper we use $^2$H NMR spectroscopy of acyl chain perdeuterated DPPE and DPPC along with de-Pakage procedures (cf. Bloom et al., 1981; Sternin et al., 1983) to compare the orientational order of the two systems. We relate the differences in order to differences in average chain cross-sectional area. It is found that the acyl chains of DPPE-d$_{62}$ have greater orientational order than for DPPC-d$_{62}$ at the same absolute and reduced temperatures. This difference in order is reflected in a smaller average chain cross-sectional area for DPPE-d$_{62}$ compared with DPPC-d$_{62}$ in the liquid-crystalline state.

**MATERIALS AND METHODS**

Palmitic acid was obtained from Sigma Chemical Co. (St. Louis, MO) and was perdeuterated by passing deuterium gas over it in the presence of a 10% palladium on charcoal catalyst (Aldrich Chemical Co., Milwaukee, WI) as described by Salmon et al., 1987. The 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) was synthesized by acylating the cadmium chloride adduct of sn-glycerol-3-phosphocholine with the anhydride of palmitic acid-d$_{16}$ (Jensen and Pitas, 1976; Mason et al., 1981). The 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine (DPPE) was synthesized by transphosphatidylating of DPPC-d$_{62}$ using phospholipase D (from Savoy cabbage) in the presence of ethanolamine (Yang et al., 1967; Pitas, 1976; Mason et al., 1981). The 1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine (DPPE-d$_{62}$) was synthesized by transphosphatidylating of DPPC-d$_{62}$ using phospholipase D (from Savoy cabbage) in the presence of ethanolamine (Yang et al., 1967; Pitas, 1976; Mason et al., 1981). The DPPE-d$_{62}$ was purified using silica gel chromatography and yielded a single spot upon TLC analysis. The DPPC and DPPE were synthesized by the method of Nagle and Wilkinson (1978; Wilkinson and Nagle, 1981; Salmon et al., 1987; Nagle and Wiener, 1988), and is given by

\[ V_{\text{chain}} = n' V_{\text{CH}} + V_{\text{CN}}. \]

In the above expression $V_{\text{CN}} = 2V_{\text{CH}}$ and $n' = n - m$ is the number of methylene segments used to calculate the projected chain length ($n'$ = 14 and 13 for the all-trans reference state is $L_0 = 1.25$ Å. From the effective chain length ($L$) an estimate can be made of the average cross-sectional area of the acyl chain ($A$) by the equation

\[ A = V_{\text{chain}}(L). \]

Here $V_{\text{chain}}$ is the volume of the acyl chain derived from the volume per methylene for each lipid (Nagle and Wilkinson, 1978; Wilkinson and Nagle, 1981; Salmon et al., 1987; Nagle and Wiener, 1988), and is given by

\[ \Delta \nu_0 = \frac{3}{2} \left( \frac{\epsilon'^2 Q}{h} \right) P_x (\cos \theta) |S_{\text{CD}}^{(0)}|. \]

where $P_x (\cos \theta) = 1$ and $(\epsilon'^2 Q/h) = 170$ kHz. In Eq. 1 the C-H bond segmental order parameters are defined by

\[ S_{\text{CD}}^{(0)} = \frac{1}{2} \left( 3 \cos^2 \beta - 1 \right), \]

in which $\beta$ is the angle between the C-H bond direction at any instant, and the brackets indicate an average over all conformations sampled on the $^2$H NMR time scale. Based on geometrical considerations the values of $S_{\text{CD}}^{(0)}$ are assumed to be negative.

The segmental order parameters can be used to estimate the average length of the acyl chain ($L$) relative to the all-trans state using a simple statistical model developed by Schindler and Seelig (1975) and later modified by Salmon et al. (1987) to yield

\[ L = L_0 \left( \frac{n - m + 1}{2} + \sum_{i=1}^{n-1} S_{\text{CD}}^{(i)} - 3S_{\text{CD}}^{(0)} \right). \]

Eq. 3 takes into account the fact that different order profiles are observed for the sn-1 and sn-2 chains of phospholipid lamellar phases in the liquid-crystalline state (Seelig and Seelig, 1974; Salmon et al., 1987). Here the index $i$ refers to the numbering of the acyl chains beginning with the ester carbon ($i = 1$) and ending with the methyl carbon ($i = n$), where $n$ is the number of carbon atoms ($n = 16$ for DPPC and DPPE). The effective acyl chain length is taken as extending from the C$_1$ carbon to the methyl carbon in the case of the sn-1 chain, i.e., $m = 2$. In the case of the sn-2 chain, the contribution from the C$_1$ carbon is neglected so that $m = 3$ (cf. Salmon et al., 1987).

The length of one carbon–carbon bond projected onto the axis of the all-trans reference state is $L_0 = 1.25$ Å. From the effective chain length ($L$) an estimate can be made of the average cross-sectional area of the acyl chain ($A$) by the equation

\[ A = V_{\text{chain}}(L). \]

Here $V_{\text{chain}}$ is the volume of the acyl chain derived from the volume per methylene for each lipid (Nagle and Wilkinson, 1978; Wilkinson and Nagle, 1981; Salmon et al., 1987; Nagle and Wiener, 1988), and is given by

\[ V_{\text{chain}} = n' V_{\text{CH}} + V_{\text{CN}}. \]

In the above expression $V_{\text{CN}} = 2V_{\text{CH}}$ and $n' = n - m$ is the number of methylene segments used to calculate the projected chain length ($n' = 14$ and 13 for the sn-1 and sn-2 chains, respectively, of DPPC and DPPE). A value of $V_{\text{CH}} = 28.0$ Å$^2$ was used in the calculations (cf. Nagle and Wilkinson, 1978).

**RESULTS AND DISCUSSION**

A representative $^2$H NMR spectrum of an aqueous dispersion of DPPE-d$_{62}$ in the L$_d$ phase is shown in Fig. 1 a, along with the corresponding de-Pakaged spectrum in Fig. 1 b. The powder-type $^2$H NMR spectrum (Fig. 1 a) consists of a series of overlapping powder patterns due to the various deuterated chain segments and is very similar to $^2$H NMR spectra for DPPC-d$_{62}$ (Davis, 1979; Salmon et al., 1987). The de-Pakaged spectrum (Fig. 1 b) corresponds to the $\theta = 0^\circ$ orientation of the bilayer normal with respect to the magnetic field. The improvement in the resolution is due to the fact that the subspectrum is spread out to the maximum extent possible which allows for easier assignment of the C-H bond order parameters, $S_{\text{CD}}^{(i)}$.

The values of $S_{\text{CD}}^{(i)}$ extracted from the de-Pakaged $^2$H NMR spectra are shown for DPPE-d$_{62}$ at two temperatures in Fig. 2 a. The chain segment assignments were
made by integrating the intensities of the de-Paked spectra and by comparison to previous results for DPPC with specifically deuterated and perdeuterated acyl chains (Seelig and Seelig, 1974, 1975; Brown et al., 1979; Blume et al., 1982a; Salmon et al., 1987). Because of the different orientations with respect to the bilayer surface, the two acyl chains of DPPC are inequivalent in the L_{α} phase, leading to separate profiles for the sn-1 and sn-2 chains (Seelig and Seelig, 1975). In the case of DPPC-d_{62} in the L_{α} phase, the quadrupolar splittings arising from the sn-1 and sn-2 chains were assigned by comparison to results for DPPC-d_{31}, in which the sn-2 chain was perdeuterated (Salmon et al., 1987). An identical pattern of splittings was evident for DPPE-d_{62} (cf. Fig. 1), which were assigned by analogy to the results for DPPC-d_{62}. The order profiles of both DPPE-d_{62} and DPPC-d_{62} show a plateau region where the order parameters are greater for DPPE-d_{62} than for DPPC-d_{62}. For this the reduced temperature is used where \( T_{\text{red}} = (T - T_m)/T_m \) and \( T_m \) is the main transition temperature (Seelig and Browning, 1978; Barry et al., 1990). At the same reduced temperatures the order is also greater for DPPE-d_{62} than for DPPC-d_{62}.

The different order profiles of these two systems are related to differences in the polar headgroup regions because the acyl chains are identical. The most obvious difference is the headgroup size and x-ray diffraction data indicate that phosphatidylethanolamines have a smaller surface area per molecule relative to phosphatidylcholines (Lis et al., 1982; Wilkinson and Nagle, 1981; McIntosh and Simon, 1986; Nagle and Wiener, 1988). Mely et al. (1975) have shown that order profiles for potassium laurate extracted from 2H NMR spectra are related to the mean area per polar headgroup. The theoretical model of Meraldi and Schlitter (1981) stresses the importance of short-range repulsive forces, which are related to the size and shape of the molecules, in governing the orientational order observed by 2H NMR in lipid systems. Therefore the smaller surface area per molecule for DPPE-d_{62} is most likely associated with the larger order parameters compared with DPPC-d_{62}; cf. also Marsh et al. (1983). The smaller cross-sectional

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**FIGURE 1** Representative 2H NMR spectra of multilamellar dispersion of DPPE-d_{62} at 69°C (\( T_{\text{red}} = 0.0419 \)) in the liquid-crystalline (L_{α}) phase. The 2H NMR powder-type spectrum corresponding to a random distribution of the lamellae is indicated in a. The de-Paked 2H NMR spectrum in b represents the \( \theta = 0^\circ \) orientation of the lamellar normal relative to the magnetic field.

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**FIGURE 2** Comparison of order profiles of DPPE-d_{62} and DPPC-d_{62} at different absolute and reduced temperatures in the L_{α} phase. The order parameters \( S_{\text{CD}} \) are plotted as a function of chain position for (a) DPPE-d_{62} at 69 and 85°C (\( T_{\text{red}} = 0.0419 \) and 0.0903) and (b) DPPC-d_{62} at 65°C (\( T_{\text{red}} = 0.0903 \)). Different order profiles are obtained for the two acyl chains and are connected by solid lines. The smaller and larger values of \( S_{\text{CD}} \) arise from the sn-1 and sn-2 chains, respectively. Note that the segmental ordering is greater for DPPE-d_{62} at all absolute and reduced temperatures compared to DPPC-d_{62}.
area available to the chains indicates that fewer gauche isomers are needed to maintain the packing near the density of liquid hydrocarbons. This same conclusion was reached by Marsh et al. (1983) when comparing DMPC-d$_{32}$ and DMPE-d$_{32}$ at the same reduced temperatures, except they found that only the plateau carbons exhibited an increase in order for DMPE-d$_{32}$. The difference between what we observe and the observations by Marsh et al. (1983) is due to the increased resolution obtained by de-Paking the spectra.

As described above, a simple diamond lattice model can be used to relate the profiles of the $S^{(0)}_{CD}$ values to the average projected length $\langle L \rangle$ and the mean cross-sectional area $\langle A \rangle$ of the acyl chains. For DPPE-d$_{62}$ at 69°C, the calculated length $\langle L \rangle$ is 12.9 Å for the sn-1 chain and 12.1 Å for the sn-2 chain. When the temperature is increased to 85°C, $\langle L \rangle$ decreases to 12.4 and 11.7 Å for the sn-1 and sn-2 chains, respectively. In Fig. 3 the calculated cross-sectional areas are compared for DPPE-d$_{62}$ and DPPC-d$_{62}$. As expected, the calculated average areas of the acyl chains are significantly less for DPPE-d$_{62}$ at all absolute and reduced temperatures compared with DPPC-d$_{62}$. Because the only difference in the two molecules is in the headgroup region, the area occupied per acyl chain must reflect the effective headgroup area at the membrane lipid/water interface. If one adds the areas of the sn-1 and sn-2 chains an estimate of the average cross-sectional area per molecule can be obtained. This technique yields a value of 71.7 Å$^2$ for DPPC-d$_{62}$ at 50°C ($T_{red} \approx 0.04$) compared with 57.6–70.9 Å$^2$ from x-ray data (Nagle and Wiener, 1988). The wide range of values from low angle x-ray diffraction studies underscores the ambiguities present when using this method in the L$_\alpha$ phase (Nagle and Wiener, 1988). A value of $\langle A \rangle = 69.5$ Å$^2$ is obtained for the interfacial area occupied per molecule of DPPE-d$_{62}$ at 69°C ($T_{red} \approx 0.04$) in the L$_\alpha$ phase, which is less than for DPPC-d$_{62}$. Although x-ray data are currently unavailable for DPPE, the smaller surface area per molecule relative to phosphatidylycerolines is in qualitative agreement with low-angle x-ray diffraction studies of egg PE, DLPE, and DMPE in the L$_\alpha$ phase (Lis et al., 1982; Wilkinson and Nagle, 1981; McIntosh and Simon, 1986; Nagle and Wiener, 1988). A reduction of ~4 Å$^2$ in the average chain cross-sectional area of DPPE-d$_{62}$ relative to DPPC-d$_{62}$ is evident at the same absolute temperature (cf. Fig. 3), from which a difference of ~8 Å$^2$ in the cross-sectional area per molecule (a 10% decrease) is suggested.

The above approach may perhaps overestimate the area per molecule because the values obtained for DPPC-d$_{62}$ are at the high end of the x-ray data range. An alternate method assumes that only the carbons nearest to the headgroup reflect the molecular area at the lipid/water interface. This would yield lower values for the mean cross-sectional area per molecule. Such an approach may be reasonable because the motions of carbon segments further down the chain are less restricted by the headgroup. On the other hand this assumption could lead to packing problems when one gets to the center of the bilayer. If we use only carbons two through ten (approximately the plateau region) in calculating the area per molecule, the values decrease to 57.4 Å$^2$ for DPPC-d$_{62}$ at 50°C and 55.4 Å$^2$ for DPPE-d$_{62}$ at 69°C. These values still reflect the smaller area per molecule for DPPE-d$_{62}$ relative to DPPC-d$_{62}$, and the differences in molecular area for these two lipids are the same as for the previous method (~10% decrease).

In summary, the differences in configurational properties of the acyl chains of DPPC-d$_{62}$ and DPPE-d$_{62}$ manifest differences in the polar headgroup region of the bilayers. These differences cause an increase in the main transition temperature for DPPE-d$_{62}$ as well as an increase in the segmental order at the same absolute and reduced temperatures compared with DPPC-d$_{62}$. The $^2$H NMR data indicate that the average area per acyl chain is significantly smaller for DPPE-d$_{62}$ than for DPPC-d$_{62}$. This difference in the chain cross-sectional area is consistent with the molecular areas determined by x-ray studies. $^2$H NMR thus provides an additional method for comparing the molecular areas of different phospholipids in the L$_\alpha$ phase. The area available for a lipid molecule in membranes is of fundamental importance in understanding their roles in biological systems. This parameter has been related to such phenomena as the formation of non-bilayer phases (including the inverted hexagonal, H$_{III}$, and cubic phases), the role of lipid diversity in biological systems, lipid-protein interactions, and diffusion/permeability in membranes (Israelachvili, 1984).
et al., 1976; Kirk et al., 1984; Wiedmann et al., 1988; DeYoung and Dill, 1988; Lindblom and Rilfors, 1989).

We are grateful to Constantine Job for expert electronics assistance, Göran Lindblom and John Nagle for helpful comments regarding the manuscript, Judith Barry for providing the computer programs used, and Ted Trouard for computer programming and for constructing the home-built $^2$H NMR probe.

Financial support of this research was provided by National Institutes of Health grants GM41413, EY03754, and RR03529.

Received for publication 13 April 1990 and in final form 13 August 1990.

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