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Phosphatidylethanolamine Enhances the Concentration-Dependent Exchange of Phospholipids between Bilayers†

William C. Wimley and T. E. Thompson*
Department of Biochemistry, University of Virginia, Charlottesville, Virginia 22908
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ABSTRACT: It has previously been demonstrated that lipid exchange between phosphatidylcholine vesicles, at higher concentrations, is characterized by a second-order concentration-dependent exchange process in addition to the first-order process operative at lower concentrations (Jones, J. D., & Thompson, T. E. (1989) *Biochemistry 28*, 129-134). Furthermore, it was demonstrated that the second-order process occurs as a result of an enhancement of the first-order desorption process, possibly resulting from attractive interactions between a potentially desorbing lipid molecule and a transiently apposed bilayer (Jones, J. D., & Thompson, T. E. (1990) *Biochemistry 29*, 1593-1600). In this work we have studied the exchange of [^{3}H]dimyristoylphosphatidylcholine (DMPC) between large vesicles of the compositions 100% DMPC, 70/30 (mol/mol) DMPC/dimyristoylphosphatidylethanolamine (DMPE), and 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/dimyristoylphosphatidylglycerol (DMPG). The second-order exchange process is enhanced by 100-fold or more in vesicles containing 30 mol % DMPE relative to 100% DMPC and is reduced or eliminated by the addition of 1.75% of the anionic lipid DMPG. These effects can be achieved by alterations in the equilibrium bilayer separation of 5 Å or less. The results are in accord with the model of Jones and Thompson and indicate that relatively low concentrations of PE in a PC bilayer can have significant effects on bilayer surface properties and on potential interactions between bilayers.

It is well established that lipid molecules exchange between bilayers via a spontaneous first-order process that involves desorption of lipid molecules from the donor bilayer surface followed by rapid diffusion through the aqueous phase to an acceptor bilayer (Martin & MacDonald, 1976; Roseman & Thompson, 1980; Doody et al., 1980; Nichols & Pagano, 1981, 1982, Massey et al., 1982; De Cuyper et al., 1983; Arvinte & Hildenbrand, 1984). Recently, however, an additional second-order concentration-dependent process was observed at lipid concentrations above about 2 mM (Jones & Thompson, 1989). This second-order process was found to be an enhancement of the first-order process, probably resulting from attractive interactions between transiently apposed bilayers and some fraction of the potentially desorbing lipid molecules (Jones & Thompson, 1990).

This model suggests that factors that influence vesicle–vesicle interactions, such as surface hydration or charge, will have significant effects on the efficiency of the concentration-dependent exchange process. For instance, phosphatidylethanolamine-containing (PE-containing) bilayers tend to be less well hydrated than phosphatidylcholine (PC) and as a result have a smaller equilibrium bilayer separation and a deeper interaction energy "well" (McIntosh & Simon, 1986; Rand & Parsegian, 1989). As a result, one would expect PE-containing bilayers to have a more efficient concentration-dependent exchange compared to PC bilayers. In the work presented here this idea was tested by studying the concentration-dependent exchange process in vesicles of pure dimyristoylphosphatidylcholine (DMPC),[1] of 70/30 (mol/mol) DMPC/dimyristoylphosphatidylethanolamine (DMPE) and of 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/dimyristoylphosphatidylglycerol (DMPG). The results indicate that the presence of 30 mol % PE in a liquid-crystalline PC bilayer has large effects on the concentration-dependent exchange of lipids between bilayers, probably through its effects on bilayer surface properties and the potential interactions between bilayers. A small amount of charged lipid in the bilayers reduces or eliminates the effects of PE.

Experimental Procedures

Vesicle Preparation. All lipids were obtained from Avanti Polar Lipids (Birmingham, AL). Purity was periodically confirmed by thin-layer chromatography. [^{3}H]DMPC (66 Ci/mol) was prepared by the method of Jones and Thompson (1989), and [^{14}C]cholesteryl oleate (56.6 Ci/mol) was obtained from New England Nuclear (Boston, MA).

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DMPG, dimyristoylphosphatidylglycerol; MLV, multilamellar vesicles; LUV, large (100-nm diameter) unilamellar vesicles prepared by extrusion; OLV, oligolamellar (350-700-nm diameter) vesicles; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); EDTA, ethylenediaminetetraacetic acid.

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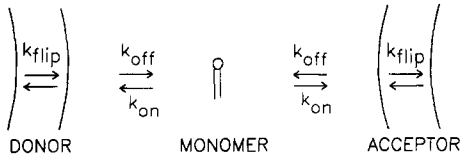

FIGURE 1: Mechanism of lipid exchange at low concentrations. The rate constants, k_{off}, k_{on}, and k_{flip} represent monomer desorption, adsorption, and transbilayer movement (flip-flop), respectively.

Lipids were dried rapidly from $CHCl_3$ in a rotary evaporator at 40 °C. Samples that showed any nonuniformity of deposition were redissolved in $CHCl_3$ and dried again to insure good mixing of the dried lipid species. After lyophilization overnight, buffer (10 mM PIPES, 50 mM KCl, 1 mM EDTA, 0.02% NaN_3, pH 7.0) was added to the flask above the phase transition temperature of the lipids, and the flask was swirled or agitated to form a rough suspension of multilamellar vesicles (MLV). To insure even solvent distribution (Mayer et al., 1985) and to decrease the amount of smaller vesicles present, the MLV solution was frozen (−20 °C) and thawed (50 °C) at least five times.

Large unilamellar donor vesicles (LUV) of 110-nm diameter were prepared by extruding the MLV suspension through three stacked 0.1-μm pore size Nucleopore polycarbonate filters with the high-pressure extrusion device obtained from Lipex Biomembranes Inc., Vancouver, BC (Hope et al., 1985; Mayer et al., 1986; Nayar et al., 1989). The details have already been described (Wimley & Thompson, 1990, 1991). The size and unilamellar nature of vesicle preparations were confirmed by quasielastic light scattering and negative-stain electron microscopy. Oligolamellar vesicle (OLV) acceptors of 350–700 nm in diameter (av ~400 nm) were prepared by extruding MLV solutions through 0.4-μm Nucleopore polycarbonate filters, followed by preparative centrifugation as described previously (Wimley & Thompson, 1991). Vesicles prepared in this way are known to be oligolamellar with between 30 and 60% of the total lipid in the outer bilayer and the remainder in the several inner lamellae (Mayer et al., 1986).

Exchange Experiments. In these experiments donors contained DMPC or DMPC/DMPE, [^{3}H]DMPC, and a trace of [^{14}C]cholesteryl oleate as a nonexchangeable marker. Acceptors contained DMPC or DMPC/DMPE. In addition, some experiments were done with vesicles containing 1.75 mol % dimyristoylphosphatidylglycerol (DMPG) to impart a slight charge on the vesicles.

In each experiment, acceptors and donors were mixed to give various total concentrations between 0.3 and 40 mM lipid at a 50/1 acceptor-to-donor ratio. At various times aliquots containing 0.1 μmol of lipid were removed and centrifuged for 4 min at 120000*g* in a Beckman Airfuge ultracentrifuge. The supernatant, which contained 50–80% of the donors and ≤5% of the acceptors, was removed, and the ^{3}H and ^{14}C were determined by liquid scintillation counting. The data are expressed as

$$^3\overline{H}_d(t) = \frac{^3H(t)/^{14}C(t)}{^3H(0)/^{14}C(0)} \quad (1)$$

where $3\overline{H}_d(t)$ is the normalized fraction of [^{3}H]DMPC remaining in the donors at time t, $^3H(t)$ and $^{14}C(t)$ are the amounts of the labels recovered at time t, and $^3H(0)$ and $^{14}C(0)$ are the amounts recovered at t = 0 (10–30 s). The data are thus explicitly corrected for the donor recovery for each point and normalized to 1.0 at t = 0.

Kinetic Analysis. The mechanism of lipid exchange between vesicles at low concentrations is given in Figure 1. The mathematics describing the exchange kinetics and data analysis have already been described in detail (Wimley, 1990; Wimley & Thompson, 1990, 1991). Briefly, one can write the differential equations that describe this mechanism with one equation for each of the five pools of lipid: the inner and outer monolayers of acceptor and donor vesicles, respectively, and monomeric lipid in the aqueous phase. Assuming that transbilayer movement, or flip-flop, is either very slow or that it is faster than or equal to intervesicular exchange, the differentials can be solved analytically (Wimley, 1990; Wimley & Thompson, 1990, 1991). When flip-flop is somewhat slower than intervesicular exchange, a different approach is required. Numerical integration of the differential equations was combined with a nonlinear least-squares curve-fitting procedure to allow the simultaneous determination of k_{flip} and k_{off} from kinetic data without requiring that the analytical solution to the differentials be known. The details of the data analysis and curve-fitting procedure have already been described (Wimley & Thompson, 1991). At higher lipid concentrations, when the second-order exchange is also operative, the lipid-exchange kinetics are qualitatively unchanged. The apparent desorption rate, in this case, is a function of the true desorption rate plus the second-order rate constant multiplied by a concentration term, as discussed below.

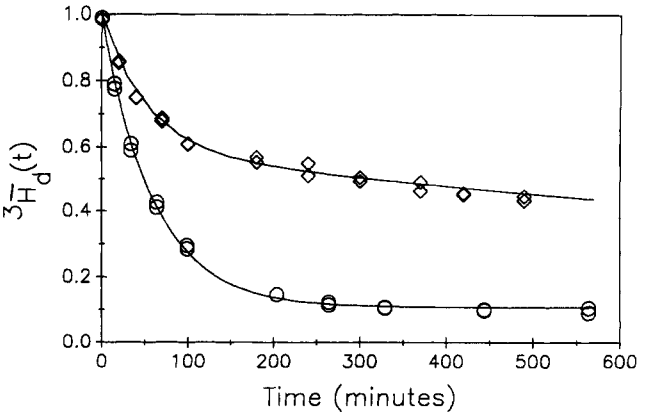

FIGURE 2: Examples of [^{3}H]DMPC exchange between vesicles at 50 °C. The circles represent exchange between 100% DMPC vesicles, and the diamonds represent exchange between 70/30 (mol/mol) DMPC/DMPE vesicles. $^3H_d(t)$ is the normalized fraction of [^{3}H]-DMPC remaining in the donor vesicles at time = t. Both of these experiments were done at 0.7 mM lipid at a 50/1 acceptor-to-donor ratio. Donors are large unilamellar vesicles and acceptors are oligolamellar vesicles prepared as described under Materials and Methods and in Wimley and Thompson (1991).

Results

Typical examples of the exchange of [^{3}H]DMPC between 100% DMPC and also between 70/30 (mol/mol) DMPC/DMPE vesicles are shown in Figure 2. Experiments with 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/DMPG were indistinguishable from 70/30 (mol/mol) DMPC/DMPE at low concentrations. Two distinct kinetic regimes are represented in these two experiments. In the bottom curve (100% DMPC) the rate of transbilayer movement is equal to or faster than the rate of intervesicular exchange, thus the kinetics are single exponential with almost all of the lipid exchanging. In the top curve of Figure 2, with vesicles of the composition 70/30 (mol/mol) DMPC/DMPE, on the other hand, transbilayer movement is slower than intervesicular exchange (Wimley & Thompson, 1991). As a result, the intervesicular exchange of the inner monolayer lipids is slow relative to the outer monolayer lipids, and the overall exchange kinetics are not single exponential. In either regime, the apparent off rate or desorption rate (k_{off}) of [^{3}H]DMPC from the bilayer surface

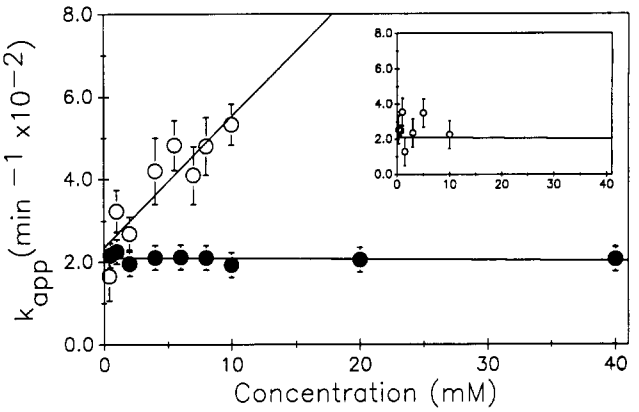

FIGURE 3: Apparent rate of [^{3}H]DMPC exchange at 50 °C with increasing lipid concentration. Filled circles represent data for [^{3}H]DMPC exchange between 100% DMPC vesicles, while open circles represent exchange between 70/30 (mol/mol) DMPC/DMPE vesicles. (Inset) The axes are identical to those in the full-size plot. In the inset the open circles represent data for [^{3}H]DMPC exchange between 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/DMPG vesicles. The horizontal lines in both panels is the best fit to the 100% DMPC data. For both plots, each point is the average of 4–6 experiments, and the uncertainties were estimated from the scatter in the values.

can be obtained as described above (Wimley & Thompson, 1990, 1991). In addition, the rate of transbilayer movement, or flip-flop (k_{flip}) can be obtained from experiments in which it is slower than intervesicular exchange, like the example shown in Figure 2 (Wimley & Thompson, 1991).

A series of experiments similar to those described above were performed at 50 °C between 0.3 and 40 mM total lipid concentration for vesicles of three different compositions: 100% DMPC, 70/30 (mol/mol) DMPC/DMPE, and 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/DMPG. The results are given in Figure 3. For [^{3}H]DMPC exchanging between 100% DMPC vesicles between 0.3 and 40 mM total lipid concentration, no dependence of the apparent exchange rate on concentration was observed. This result is not necessarily in contrast with the concentration dependence of phospholipid exchange observed by Jones and Thompson (1989, 1990) using small sonicated vesicles because of the differences in the collisional frequencies of the small vesicles used in those studies compared to the large vesicles used here. This point will be discussed later.

The inclusion of 30 mol % DMPE causes a distinct concentration dependence of the rate of [^{3}H]DMPC exchange between vesicles as shown in Figure 3. The apparent exchange rate can be expressed $k_{app} = k_{off} + k_c[A + D]$, where k_{off} is the first-order off-rate constant, k_c is the second-order rate constant, and A and D are the concentration of acceptor and donor lipid, respectively.[2] From the slope and intercept of the 70/30 (mol/mol) DMPC/DMPE data in Figure 3 $k_{off} = 2.11 \pm 0.3 \times 10^{-2}$ min^{-1} and $k_c = 3.46 \pm 0.3 \times 10^{-3}$ min^{-1} mM^{-1}, while for the 100% DMPC data $k_{off} = 2.07 \pm 0.2 \times 10^{-2}$ min^{-1} and $k_c \leq 5 \times 10^{-5}$ min^{-1} mM^{-1}. The inset of Figure 3 contains similar data for vesicles of the composition 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/DMPG, which is essentially identical to 70/30 (mol/mol) DMPC/DMPE except that 1.75 mol % of the DMPC has been replaced by the anionic lipid DMPG to impart a slight charge on the bilayers. In this case $k_{off} = 2.70 \pm 0.8 \times 10^{-2}$ min^{-1} and $k_c \leq 2 \times 10^{-4}$ min^{-1} mM^{-1}. These data indicate that a small amount of charge can reduce or eliminate the concentration-dependent exchange of lipids at the concentrations used in these experiments. For both sets of data with 30 mol % DMPE, the rate of transbilayer movement was found to be $2.15 \pm 0.9 \times 10^{-3}$ min^{-1} with no dependence on concentration. This value is very similar to the value of $2.52 \pm 0.3 \times 10^{-3}$ min^{-1} already reported for the transbilayer diffusion rate of DMPC in 68.25/30/1.75 (mol/mol/mol) DMPC/DMPE/DMPG vesicles at low concentrations (Wimley & Thompson, 1991).

Discussion

Recently, Jones and Thompson (1989, 1990) have demonstrated that a spontaneous second-order concentration-dependent or collisionally mediated lipid-exchange process occurs between phosphatidylcholine bilayers in addition to the usual spontaneous first-order process. Only at high lipid concentrations, however, does the second-order process become quantitatively important in the overall lipid-exchange kinetics. In addition, it was demonstrated that the second-order process results from an enhancement of the first-order process. Jones and Thompson suggested that the mechanism of the collisionally mediated exchange was a decrease in the activation energy barrier to desorption of a fraction of the potentially desorbing lipid molecules caused by the van der Waals attraction between the potentially desorbing molecule and a nearby bilayer. The fraction of affected molecules is dependent on the vesicle concentration, and thus only at high concentrations are enough molecules influenced to affect the overall exchange kinetics. It was also demonstrated that dehydration and physical contact between bilayers are not involved. Instead, transient apposition of the fully hydrated surfaces at approximately the equilibrium separation of the bilayers is sufficient.

If this model is correct, then the efficiency of the collisionally mediated exchange process will be critically dependent on the equilibrium bilayer separation and on the depth of the interaction energy minimum, which will influence the lifetime of the collisional complex. Phosphatidylethanolamines (PE) are known to be less hydrated than phosphatidylcholines (PC) and have a smaller equilibrium bilayer separation with a deeper potential energy well (McIntosh & Simon, 1986; Rand & Parsegian, 1989). As a result, PE-containing vesicles can become more closely apposed than pure PC vesicles and vesicle interactions may be longer lived. Thus, based on the model of Jones and Thompson (1989, 1990), one can predict that the concentration-dependent exchange process should be more efficient in PE-containing vesicles than in PC vesicles. This prediction is confirmed in the work presented here; the efficiency of the collisionally mediated exchange is enhanced at least 100-fold in DMPC bilayers by the addition of 30 mol % DMPE.

In order to compare the results obtained with different lipids, it is useful to compare the ratio of k_{off}/k_c, since this gives a measure of the enhancement of the first-order exchange process by the second-order process even when the absolute values of k_{off} are widely different. Additionally, in order to compare the inherent efficiency of the concentration-dependent exchange process between experiments done at different temperatures or with vesicles of different sizes, one needs to correct for the effect of these factors on vesicle collision frequency.

[2] For donors and acceptors of different sizes this is only strictly true if the lipid molecules involved in the second-order exchange transfer directly into the apposed acceptors. As discussed below this is probably a reasonable assumption. For equally sized donors and acceptors this equation is correct for direct transfer as well as for collisionally enhanced desorption of monomeric lipid molecules into the aqueous phase followed by random diffusion. Jones and Thompson (1989) derived $k_{app} = k_{off}[A/(A + D)] + k_cA$ for the initial rate of lipid exchange between vesicles. This is also correct for either of the above mechanisms.

Table I: Efficiency of Concentration-Dependent Phospholipid Exchange

lipid[a]	vesicle type[b]	T (°C)	k_{off}/k_c	$(k_{off}/k_c)_N$
POPC[c]	POPC SUV	45	9.8	290[d]
POPC[c]	POPC LUV	45	189	170
POPC[c]	POPC SUV	30	13	280
DMPC[c]	POPC SUV	30	31	670
DMPC[e]	70/30 DMPC/DMPE	50	7.6	0.55
DMPC[e]	100% DMPC	50	>800	>60
DMPC[e]	68.25/30/1.75 DMPC/DMPE/DMPG	50	>100	>8

[a] The exchanging lipid. [b] SUV, small unilamellar vesicles prepared by sonication; LUV, large unilamellar vesicles prepared by extrusion. [c] Taken from Jones and Thompson (1990). [d] k_{off}/k_c normalized to the reference conditions of 50 °C and 110-nm diameter LUV by $(k_{off}/k_c)_N = (k_{off}/k_c)(f_{AD}/f^0_{AD})$, where f^0_{AD} is the frequency of acceptor donor collisions per donor vesicle at the reference conditions and f_{AD} is the frequency of acceptor donor collisions per donor vesicle at the experimental conditions; f_{AD} is proportional to $1/L_A(D_A + D_D)R_{AD}$, where L_A is the number of lipid molecules in each acceptor vesicle, D_A and D_D are the acceptor and donor diffusion coefficients, respectively, and R_{AD} is the sum of acceptor and donor radii (Jones & Thompson, 1990). From quasielastic light scattering and negative-stain electron microscopy, the following vesicle diameters are used: SUV, 25 nm; LUV, 110 nm; OLV, 400 nm; and the following estimates for L_A are used: SUV, 3000; LUV, 1×10^5; OLV, 2×10^6 lipids/vesicle. The uncertainties in these estimates and in the use of a hard-sphere treatment of vesicle collisions generate an uncertainty in $(k_{off}/k_c)_N$ of a factor of 2 or more. [e] This work. Donor vesicles are 110-nm LUV, and acceptor vesicles are approximately 400-nm oligolamellar vesicles prepared as described earlier.

Given in Table I are the ratios of k_{off}/k_c from the present work and from the data of Jones and Thompson (1990) covering a wide range of experimental conditions. The final column gives the values of k_{off}/k_c normalized to the reference conditions of 50 °C and 110-nm diameter large unilamellar vesicles. These data indicate the relative efficiency of the concentration-dependent exchange process. In agreement with the conclusions above, the efficiency of collisionally mediated lipid exchange is 100–1000-fold greater in the presence of 30 mol % DMPE compared to DMPC or POPC vesicles. These results indicate that the interactions between a potentially desorbing lipid molecule and a nearby vesicle are increased by at least several kcal/mol in the presence of 30 mol % DMPE.

The activated, or highest free energy, state of a desorbing lipid molecule probably occurs when it is almost completely in the aqueous phase with only a small part of its acyl chains anchoring it to the bilayer (McLean & Phillips, 1984; Wimley & Thompson, 1990). This means that a DMPC molecule in the activated state can extend as much as 20 Å away from the bilayer and thus would be affected by an apposed bilayer if the activated lipid molecule exists between bilayers separated by 25 Å or less. This distance is very similar to the equilibrium separation between bilayers in DMPC multilamellar vesicles (Rand & Parsegian, 1989). The potential attractive interactions between a lipid monomer and a bilayer depend on the inverse of the distance between them, sharply increasing to values greater than 1 kcal/mol only at very small separations (Parsegian 1975; Jones & Thompson, 1990). Therefore to achieve a decrease of several kcal/mol, the effect of 30 mol % DMPE must be to decrease the average distance between activated lipid molecules and apposed bilayers so that they are nearly in physical contact. This could be achieved by a decrease in the average interbilayer separation, in the collisional complex, of 5 Å or less to reasonable bilayer separations of about 20 Å.

The effect of charge on the interactions between vesicles is twofold. First, electrostatic repulsions will increase the equilibrium separation between bilayers in the collisional complex and will decrease the depth of the interaction energy minimum (Parsegian & Rand, 1983). As discussed above, a change of 5 Å or less in the equilibrium bilayer separation can account for the observed effects. Second, long-range electrostatic interactions will decrease the frequency of collisions between charged vesicles, which will also decrease the apparent efficiency of the second-order exchange process. A bilayer containing 1.75 mol % of DMPG, in 50 mM KCl, has an electrostatic membrane surface potential of about −9 mV (Cevc, 1990). Under these conditions, the Debye screening length of the counterion atmosphere near the bilayer is about 14 Å. Biological membranes have been reported to have a surface potential of −15 to −30 mV under physiological conditions (Cevc, 1990), where the Debye length is roughly 7 Å. Therefore, the effects of charge on phospholipid transfer observed in our system are probably similar to the effects of charge on the transfer of phospholipids between the membrane bilayers of cellular organelles. However, many membranes may have laterally segregated domains of low charge density that could be involved in interactions between bilayers. In addition, apposition of charged membranes can be induced by cations (Ohki et al., 1982) or proteins (Creutz, 1981). Under these conditions, spontaneous lipid exchange between apposed membrane bilayers and its control by bilayer composition may be an important biological process. Finally, Jones and Thompson (1990) have shown that the magnitude of the relative enhancement of lipid exchange at high vesicle concentrations is different for different phospholipids and is very small for cholesterol. Therefore, even controlled transfer of specific lipid species between apposed biological membranes is possible.

Acknowledgments

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Registry No. DMPC, 18194-24-6; DMPE, 998-07-2; DMPG, 61361-72-6.

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Bilayers of Arachidonic Acid Containing Phospholipids Studied by ^2H and ^{31}P NMR Spectroscopy†

Kannan Rajamoorthi and Michael F. Brown*
Department of Chemistry, University of Arizona, Tucson, Arizona 85721
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ABSTRACT: The configurational properties and dynamics of the arachidonic acyl chains of phospholipid bilayers have been investigated for the first time by solid-state ^2H NMR techniques, with the goal of achieving a better understanding of the biological roles of polyunsaturated phospholipids. Vinyl perdeuterated arachidonic acid ($20:4^{\Delta5,8,11,14}$-d_8) was prepared from eicosatetraynoic acid (ETYA) and was esterified with 1-palmitoyl-*sn*-glycero-3-phosphocholine to yield 1-palmitoyl-2-vinylperdeuterioarachidonoyl-*sn*-glycero-3-phosphocholine [(16:0)(20:4-d_8)PC]. ^{31}P NMR spectra of aqueous dispersions of (16:0)(20:4-d_8)PC as well as 1-perdeuteriopalmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine [(per-^2H-16:0)(20:4)PC] were characteristic of the lamellar liquid-crystalline state. The dispersions had similar ^{31}P chemical shift anisotropies, with little apparent motional averaging of the lineshapes due to macroscopic reorientation of liposomes or lateral diffusion of phospholipids about their curved surfaces. Comparison to other phosphatidylcholines indicated that both samples comprised the fully hydrated L_α phase plus excess water. However, the dispersion of (16:0)(20:4-d_8)PC yielded relatively narrow powder-type ^2H NMR spectra, compared to (per-^2H-16:0)(20:4)PC in the liquid-crystalline state. The differences in the ^2H NMR powder patterns thus reflect differences in the configurational properties of the polyunsaturated *sn*-2 arachidonic acyl chain compared to the saturated *sn*-1 palmitic chain. When the powder-type ^2H NMR spectra of the (16:0)(20:4-d_8)PC bilayer were dePaked ($\theta = 0°$), they showed three kinds of deuterons upon integration: one with a large splitting (≈25-35 kHz), two with intermediate splittings (≈10-15 kHz), and the remainder with smaller splittings (≈0.3-5 kHz). The residual quadrupolar couplings of the vinylic >C=C< deuterons were less than those of most C^2H_2 groups of the saturated acyl chain. It is concluded that some of the >C=C< segments are inequivalent and exhibit differences along the arachidonic acyl chain, as found previously for the C^2H_2 groups of the polymethylene chains of saturated phospholipid bilayers. In addition, the ^2H spin-lattice relaxation rates, R_{1Z}, of the vinylic >C=C< deuterons of the *sn*-2 arachidonic acyl chain were larger relative to their quadrupolar splittings than for most C^2H_2 segments of the saturated *sn*-1 chain. Simple models for interpretation of the findings are briefly mentioned and discussed. The results indicate clearly that the structural and dynamic properties of polyunsaturated acyl chains differ from those of saturated and monounsaturated chains in phospholipid bilayers.

^2H NMR[1] spectroscopy provides a useful and general means of obtaining information regarding the orientational order and motion of individual segments of the hydrocarbon chains of lipid bilayers in the liquid-crystalline state (Seelig & Seelig, 1977; Seelig & Browning, 1978; Brown et al., 1979, 1983; Brown, 1979, 1982, 1984; Paddy et al., 1985; Salmon et al., 1987; Thurmond et al., 1991). At present, ^2H NMR spectroscopy has been used mainly for investigations of bilayers containing saturated and monounsaturated acyl chains (Seelig & Seelig, 1974; Seelig & Waespe-Šarčevič, 1978; Rance et

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* Author to whom correspondence should be addressed.

[1] Abbreviations: BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; ETYA, eicosatetraynoic acid; GC, gas-liquid chromatography; GC-MS, gas chromatography-mass spectrometry; HPLC, high-pressure liquid chromatography; NMR, nuclear magnetic resonance; ppm, parts per million; TLC, thin-layer chromatography; (per-^2H-16:0)(20:4)PC, 1-perdeuteriopalmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; (16:0)(20:4-d_8)PC, 1-palmitoyl-2-vinylperdeuterioarachidonoyl-*sn*-glycero-3-phosphocholine; (16:0)(22:6-d_{12})PC, 1-palmitoyl-2-vinylperdeuteriodocosahexaenoyl-*sn*-glycero-3-phosphocholine; (18:1)(18:1-d_2)PC, 1-oleoyl-2-vinylperdeuteriooleoyl-*sn*-glycero-3-phosphocholine.

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