Influence of Phospholipid Chirality on Nearest-Neighbor Interactions within Fluid Bilayers

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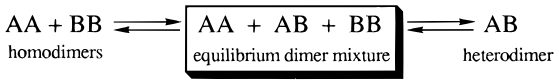
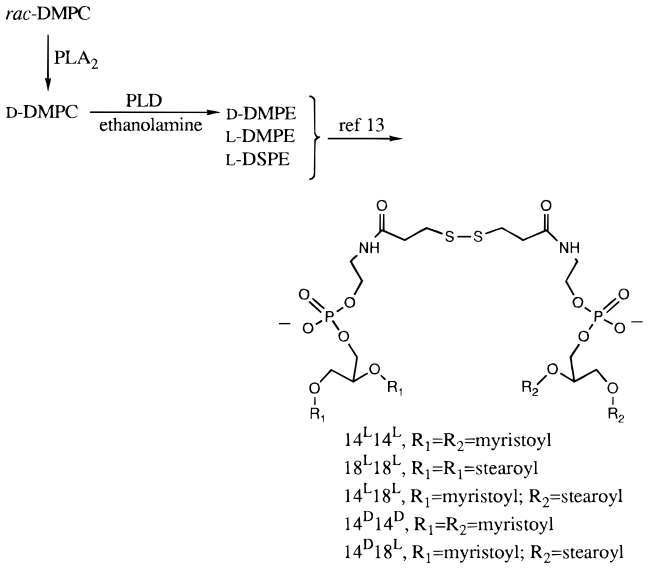
Phospholipids serve as essential bilayer-forming material for all biological membranes.[1] The fact that phospholipids are inherently chiral due to their *sn*-2-carbon atom, and that only one enantiomeric form exists in nature, raises the intriguing possibility that stereospecific interactions between them and between other chiral membrane components (i.e., sterols and globular proteins) may contribute to their overall two-dimensional organization. Previous attempts that have been made to clarify the effects of chirality on the packing behavior of phospholipids have yielded mixed results. In some cases, chiral effects have been noted; in other cases, no effects were observed.[2−8] In the most thorough study to date, no stereospecific interactions could be detected between enantiomeric and racemic forms of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) through the use of differential scanning calorimetry, high-field NMR spectroscopy, and a variety of monolayer methods.[9,10] More recent studies, however, have revealed that chirality can have a profound effect on the supramolecular behavior of phospholipids. For example, enantiomers of 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine have been shown to form novel tubules, apparently as a result of "chiral packing" of the membrane—a process that relies upon individual stereospecific interactions between neighboring phospholipids.[11] In addition, chiral solid domains of DPPC have been visualized by epifluorescence optical microscopy in the monolayer state.[12] For both cases, chiral effects were observed only in the gel phase.

In this paper, *we provide compelling evidence that phospholipid chirality can influence the interactions between nearest-neighbors in the fluid bilayer state*. The method of detection that we have used is chemical in nature and is based on a nearest-neighbor recognition (NNR) analysis.[13−15] In a typical NNR experiment, two phospholipids (A and B) are converted into exchangeable, disulfide-linked homodimers (AA and BB) and a heterodimer (AB). Subsequent vesicle formation, using



an equimolar mixture of the two homodimers, followed by monomer interchange, leads to an equilibrium mixture. To ensure that an equilibrium point has been reached, equilibration is also carried out using vesicles prepared from pure heterodimer. When the resulting dimer composition is statistical (i.e., when the molar ratio of heterodimer to each homodimer is 2.0), and when transmembrane exchange of the phospholipids ("flip-flop") is negligible, this finding establishes that (i) the monomeric components are randomly distributed throughout the membrane and (ii) there is no thermodynamic preference for one phospholipid to be a nearest-neighbor of another, i.e., there is complete mixing of the phospholipids. When homodimers are favored, however (i.e., when the molar ratio is less than 2.0), the dimers may be either *randomly or nonrandomly* arranged. In the former case, dimer stability is dominated by intramolecular interactions between monomer units. In the latter case, intramolecular and intermolecular interactions contribute, similarly, to dimer stability, and NNR reflects the presence of lateral heterogeneity. Experimentally, one can distinguish between these two situations by introducing a nonexchangeable diluent into the membrane, provided that it functions as a mixing agent for A and B and that it does not alter the phase properties of the bilayer. Specifically, a laterally heterogeneous state is indicated by the reduction or elimination of NNR.[14] In the limiting case, where the molar ratio is 0 (absence of heterodimers) and the introduction of a diluent promotes heterodimer formation, complete segregation of the phospholipids is indicated.

Scheme 1



To probe the effects of chirality on nearest-neighbor interactions in fluid bilayers, we have examined the mixing behavior of exchangable monomer units derived from 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (L-DMPE) and 1,2-stearoyl-*sn*-glycero-3-phosphoethanolamine (L-DSPE) and have compared them with that of exchangable monomers formed from 2,3-dimyristoyl-*sn*-glycero-1-phosphoethanolamine (D-DMPE) and L-DSPE. Scheme 1 shows the specific dimers that were used in this study. For purposes of convenience, we refer to homodimers derived from L-DMPE, D-DMPE, and L-DSPE as 14^L14^L, 14^D14^D, and 18^L18^L, respectively, where the base number represents the total number of carbon atoms that are present in each of the fatty acid chains of the phosphoethanolamine precursor and where the superscript indicates the enantiomeric form. The corresponding heterodimers are thus designated as 14^L18^L and 14^D18^L. The bridging disulfide moiety present provides a basis for monomer exchange via thiolate−disulfide interchange.[16]

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Table 1. Nearest-Neighbor Recognition within Fluid Phospholipid Membranes[a]

lipid components	heterodimer/homodimer[b]
14^{D} + **18**^{L}	1.79 ± 0.03
14^{L} + **18**^{L}	2.06 ± 0.06 (1.98 ± 0.05)[c]
14^{D} + **18**^{L} + 50 mol % of DPPC[d]	1.78 ± 0.06
14^{D} + **18**^{L} + 50 mol % of 1,3-DPPC[e]	1.98 ± 0.06

[a] The gel to liquid-crystalline phase transition temperatures for **14**^{L}**14**^{L}, **14**^{L}**18**^{L}, and **18**^{L}**18**^{L} are 22.7, 33.9, and 55.4 °C, respectively.[13] All nearest-neighbor recognition experiments were carried out at 60 °C; chemical equilibrium was generally reached in ca. 3 h. [b] Molar ratio of heterodimer to each homodimer ± two standard deviations from the mean. [c] See ref 13. [d] 50% of the phosholipid monomer units have been replaced by DPPC. [e] 50% of the phosholipid monomer units have been replaced by 1,3-DPPC.

Specific synthetic methods that were used to prepare 14^{L}14^{L}, 14^{L}18^{L}, and 18^{L}18^{L} have previously been described;[13] stereoisomers 14^{D}14^{D} and 14^{D}18^{L} were prepared using analogous procedures except that D-DMPE was used as starting material. The latter was obtained by partial enzymatic hydrolysis of a racemic mixture of DMPC with phospholipase A_2, followed by treatment of the recovered D-DMPC with ethanolamine in the presence of phospholipase D.[17] This synthesis relies upon the ability of phospholipase A_2 to hydrolyze only the L-form of the phospholipid.[9] The optical rotations that were measured for D-(−)-DMPC and D-(−)-DMPE were $[\alpha]^{25}_{D}$ −7.25 [*c* 4.77, $CHCl_3/CH_3OH$ (1:1, v/v)] and $[\alpha]^{25}_{D}$ −7.33 (*c* 3.43, $CHCl_3$), respectively; corresponding rotations that were observed for commercially available L-(+)-DMPC and L-(+) DMPE (Avanti Polar Lipids) were $[\alpha]^{25}_{D}$ +7.07 [*c* 5.95, $CHCl_3/CH_3OH$ (1:1, v/v)] and $[\alpha]^{25}_{D}$ +7.31 (*c* 3.75, $CHCl_3$), respectively. All of the protocols that were used for vesicle formation, dimer equilibration, and HPLC analysis have previously been described.[12,13]

When fluid bilayers, composed of dimers that were derived from 14^{L} and 18^{L} monomer units, were chemically equilibrated at 60 °C, a heterodimer/homodimer ratio of 2.06 ± 0.06 was observed (Table 1). These results are in good agreement with those previously reported.[13,14] In sharp contrast, equilibration of analogous membranes that were derived from 14^{D} and 18^{L} monomer units afforded a heterodimer/homodimer ratio of 1.79 ± 0.03. In an effort to determine whether or not the observed NNR reflects the presence of lateral heterogeneity, dilution experiments were performed using a conventional phospholipid (DPPC) having a melting temperature (T_m = 41.5 °C) that lies between those of exchangeable homodimers. In brief, replacement of 50% of the exchangeable phospholipids with DPPC did not enhance the mixing of the monomers, i.e., the heterodimer/homodimer ratio was 1.78 ± 0.06. The fact that DPPC does not reduce the level of NNR within equilibrated bilayers derived from 14^{D} and 18^{L} implies that DPPC is distributed, heterogeneously, throughout such membranes. In sharp contrast, similar dilution with an achiral analog, 1,3-dipalmitoyl-*sn*-glycero-3-phosphocholine (1,3-DPPC, T_m = 37.5 °C), led to a random distribution of dimers.[19] This result indicates that in the absence of this diluent, the lateral distribution of the 14^{D} and 18^{L} units is heterogeneous.[20]

Taken together, these results provide the first compelling evidence that phospholipid chirality can influence the two-dimensional structure of *fluid* bilayers. Although the magnitude of this effect for the 14^{D} and 18^{L} system described herein is modest (a heterodimer/homodimer ratio of 1.79 ± 0.03 corresponds to a $\Delta G°$ of 186 ± 61 cal/mol at 60 °C), the fact that natural membranes are rich in other chiral components (sterols and globular proteins) suggests that chirality, in general, may play an important role in defining the lateral organization of biological membranes, especially if chiral, structural, and compositional effects on nearest-neighbor interactions are cooperative. In a broader context, the present findings highlight the need for taking chirality into account in the design of new bilayer-forming and bilayer-disrupting surfactants, where lateral packing and/or supramolecular structure are of primary importance, e.g., in the design of new membrane materials and novel drugs that operate via membrane disruption. Efforts aimed at defining the influence of other chiral components on nearest-neighbor interactions within phospholipid bilayers are continuing in our laboratories.

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Supporting Information Available: Experimental procedure for the digestion of large unilamellar vesicles composed of an equimolar mixture of homodimers of 14^{D} and 18^{L} by phospholipase A_2 and one figure showing plots of the perecent of each homodimer that remains as a function of time of exposure to phospholipase A_2 (3 pages). See any current masthead page for ordering and Internet access instructions.

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(16) Experimentally, a small percentage of the thiol-monomers is generated via partial reduction with dithiothreitol.

(17) The enzymatic procedures that were used to isolate D-DMPC from a racemic mixture were similar to those previously described.[18] Phospholipase D, which was used in the transphosphatidylation reaction, was freshly extracted from cabbage. In a typical transphosphatidylation reaction, D-(−)-DMPC (131.9 mg, 195 μmol) was dissolved in a mixture that was made from 12 mL of ether and 3 mL of chloroform. To the resulting solution was added 8 mL of 88 mM acetate buffer (pH 5.4) that was 62.5 mM in $CaCl_2$ and 245 mM in ethanolamine hydrochloride. After the mixture was heated to 45 °C with stirring, 3 mL of a phospholipase D solution [made from 1.0 g of crude enzyme in 3 mL of 80 mM acetate buffer (pH 5.4) that was 40 mM in $CaCl_2$] was then added. The mixture was stirred for 12 h, followed by addition of another 1.0 g of crude enzyme (dissolved in 2 mL of the same buffer). After an additional 10 h of stirring at 45 °C, the mixture was cooled to room temperature and concentrated under reduced pressure, and the residue was then extracted with 50 mL of $CHCl_3/CH_3OH$ (4/1,v/v). Subsequent filtration, concentration under reduced pressure, and chromatography [silica gel, $CHCl_3/CH_3OH$/2 M NH_4OH, 40:10:1], afforded 66.1 mg (54%) of D-(−)-DMPE.

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(19) 1,3-DPPC was purchased from Fluka Chemika-BioChemika and used as obtained.

(20) Further evidence in support of lateral heterogeneity was obtained by establishing that neither of the homodimers favors the inner or outer monolayer leaflet of the bilayer, i.e., there is no driving force for transmembrane asymmetry in these systems. Thus, a dispersion of large unilamellar vesicles (ca. 9000 Å diameter, dynamic light scattering) was prepared from an equimolar mixture of homodimers of 14^{D} and 18^{L} and subjected to phospholipase A_2 at 60 °C. Analysis of the nondigested dimers as a function of time showed that the homodimer of 14^{D} was completely inert toward enzymatic hydrolysis. In sharp contrast, 50% of the homodimer of 18^{L} was readily hydrolyzed. Since the number of phospholipids that are present in the inner monolayer of a large unilamellar vesicle is the same as that which is present in the outer monolayer, these findings indicate that both homodimers are evenly distributed between both halves of the bilayer and that the observed NNR reflects the same degree of lateral heterogeneity within each leaflet.