Chain Length Dependent Thermodynamics of Saturated Symmetric-Chain Phosphatidylcholine Bilayers

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ABSTRACT

A molecular interpretation for the chain length dependent thermotropic behavior of saturated symmetric-chain phosphatidylcholine bilayers is proposed. It is suggested that the bilayer interface region and conformationally inequivalent terminal ends of the fatty acyl chains perturb the packing associations of the rest of the hydrocarbon chains in the gel phase of the bilayer. These perturbing effects, which are seen to increase with decreasing acyl chain length, have been quantitatively defined by a perturbation parameter, P. The thermodynamic parameters of the thermal phase transition of these phosphatidylcholines are found to be linearly correlated to P and these linear relationships can be used to predict the minimum number of carbon atoms in the acyl chain necessary in order for a bilayer phase transition to occur.

Of the properties which characterize synthetic saturated phospholipids in model membranes, the thermally induced gel ⟷ liquid crystalline phase transition has been the one most rigorously investigated, experimentally as well as theoretically (1-7). These studies have lead to a better understanding of the molecular structure of phospholipid molecules in the bilayer, and moreover, the phase transition appears to be critical to the proper function of biological membranes (8).

It has long been established that, for saturated symmetric-chain diacylphosphoglycerides, the main transition temperature and associated thermodynamic parameters, such as transition enthalpy and entropy, are dependent on the length of the lipid's fatty acyl chains (1). For the homologous series of saturated symmetric-chain phosphatidylcholines (PC), e.g., the transition temperature and other thermodynamic parameters (ΔS, ΔH, ΔV) increase with increasing acyl chain length. However, recent studies (5) have shown that these thermodynamic parameters are a curvilinear, rather than a linear, function of the chain length of these PC. This behavior contrasts with that of bulk hydrocarbons, corresponding in length to the PCs' acyl chains, for which thermodynamic parameters have been shown to be a linear function of hydrocarbon chain length (6). In the literature, there are a number of empirical methods to linearly correlate the melting behavior of diacylglycerides with acyl chain length (7,9). These methods are useful but are based purely on curve-fitting techniques rather than on molecular interpretation.

In this communication, the PC fatty acyl chains will be viewed as consisting of three regions of different structural order (Fig. 1). The bilayer interface (region 1) and conformationally inequivalent terminal ends of the acyl chains (region 3) are postulated as perturbing the packing associations of the rest of the hydrocarbon chains (region 2) in the gel phase of the bilayer. These perturbing effects, which increase with decreasing acyl chain length, will be quantitatively defined by a perturbation parameter, P. The thermodynamic parameters of the thermal phase transition of saturated symmetric-chain PC will be shown to be a linear function of P. These linear relationships can, in turn, be used to predict the minimum number of carbon atoms in the acyl chain that is necessary for a bilayer phase transition to occur.

THERMODYNAMIC DATA OF SATURATED SYNTHETIC PHOSPHOTIDYLCHOLINES IN BILAYERS

Listed in Table 1 are the literature values for the main transition temperature (T_m) and the observed transition enthalpy (ΔH) for a series of even-numbered PC with saturated symmetric

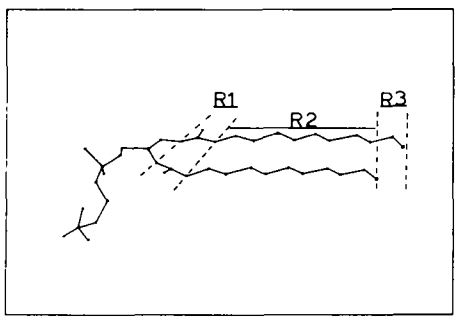

FIG. 1. Schematic diagram of 1,2-dilaurylphosphatidylcholine showing the partitioning of the fatty acyl chains into the structural regions R1, R2 and R3. Note the conformational inequivalence of the acyl chains which results in the *sn*-2 acyl chain being 1.5 carbon bond lengths shorter than the *sn*-1 acyl chain.

TABLE 1

Thermodynamic Parameters of Saturated Symmetric-Chain Phosphatidylcholines[a]

Parameter	1,2-Diacylphosphatidylcholine					LCC[b]	Linear equation
	C_{22}	C_{18}	C_{16}	C_{14}	C_{12}		
P	9.1	11.5	13.2	15.6	18.9	–	–
T_m (C)	75	55	41	24	-1.8	0.999	-7.77(P)+145
ΔH (kcal/mol)	14.8	10.6	8.6	5.4	1.7	0.994	-1.31(P)+26.4
ΔS_H(e.u./mol)	83.6	68.4	60.8	53.2	45.6	–	–
ΔS (e.u./mol)	42.5	32.3	27.4	18.2	6.27	0.998	-3.66(P)+75.3
ΔV (L/mmol)	–	3.56	2.72	1.83	–	0.993	-0.419(P)+8.33
$\Delta V/CH_2$ (mL/mol)	–	0.99	0.85	0.65	–	1.000	-0.083(P)+1.94

[a]C_n refers to the acyl chain length of the 1,2-diacylphosphatidylcholines. The values for T_m and ΔH are taken from ref. 6 for C_{22}, from refs. 5, 10 for C_{16}, and from ref. 5 for the rest of the phosphatidylcholines. Values for ΔV and $\Delta V/CH_2$ are taken from Nagel and Wilkinson (7). See the text for explanations of ΔS_H, ΔS and P.

[b]LCC is the linear correlation coefficient for a fit of the indicated parameter with the perturbation parameter, P.

acyl chains which undergo the gel → liquid crystalline phase transition. The values for dibehenoyl PC are from Phillips et al. (6), those for dipalmitoyl PC are the average of the values reported by Mabrey and Sturtevant (5) and Albon and Sturtevant (10), and the remaining values are from Mabrey and Sturtevant (5). The transition entropy is derived from the Clausius equality as: $\Delta S = \Delta H/T_m$, assuming an equilibrium first order transition (7). In Figure 2, this observed transition entropy (solid line) is plotted against fatty acyl chain length (N). The dashed line in Figure 2 is the fusion entropy of bulk hydrocarbons corresponding in length to the fatty acyl chains of the PC. This value is obtained as: ΔS_H = 1.9 e.u./mol × (2N), based on the value of 1.9 e.u./mol/carbon unit reported for the fusion entropy of bulk *n*-hydrocarbons which undergo the α → melt rotomeric chain disorder transition (6,11). Figure 2 reveals that the observed transition entropy in lipid bilayers is less than that for the corresponding bulk hydrocarbons by at least a factor of two. The smaller change in ΔS for the hydrocarbon chain in lipid bilayers is expected, since one end of the chain is in an ester linkage to the glycerol backbone of the phospholipid molecule, resulting in a decrease in motional freedom of the acyl chains relative to the free fatty acids (12). Interestingly, Figure 2 also shows that the observed change in ΔS for phospholipids in bilayers is not a linear function of the acyl chain length.

PHOSPHOLIPID STRUCTURE IN BILAYERS

The fatty acyl chains have been partitioned into three structural regions as diagrammed in Figure 1.

Region 1

Region 1 (R1) has been termed the bilayer interface (12) and consists of the carbonyl ester linkage and α carbons of both fatty acyl chains. The carbonyl-oxygen double bond and partial double bond character of the ester bond, which arises by resonance, imparts a coplanar structure to the interface elements (13). This structural planarity is similar to that observed for the peptide bond of proteins. Moreover, the C-H bond at the attachment site of the secondary ester to the glycerol backbone is arranged so as to be synplanar to the *sn*-1 acyl ester

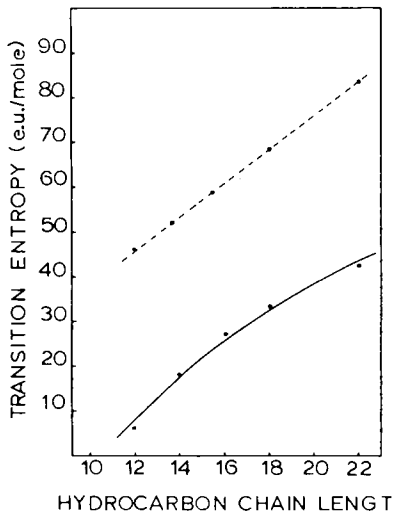

FIG. 2. Plot of the transition entropy of even saturated symmetric-chain phosphatidylcholines as a function of fatty acyl chain length. See text for an explanation of ΔS (—) and ΔS_H (-----).

linkage (see Fig. 1, ref. 12). The planarity of the bilayer interface would be expected to result in a highly restricted motion for the elements within this region. Indeed, both ^{13}C-NMR spin lattice relaxation times (14) and ^{2}H-NMR order parameters (15,16) indicate that this region possesses the smallest degree of segmental motion of all of the carbon bonds along the acyl chain. The relative structural rigidity of the bilayer interface, which persists in the liquid-crystalline phase, would lead us to predict that the acyl chain carbons within region 1 do not directly participate in the bilayer thermal phase transition.

Region 3

Although X-ray diffraction techniques have been employed to investigate the bilayer structure of membranes for some time, the crystalline structure of saturated symmetric PC at atomic resolution was reported only recently (17). X-ray data indicate that the initial segment of the *sn*-2 fatty acyl chain extends perpendicular to the *sn*-1 chain but the chain bends abruptly at the C(2) atom so that the rest of the *sn*-2 chain runs parallel to the linear *sn*-1 fatty acyl chain. Because of the abrupt bend, the terminal methyl groups of the two alkyl chains are not in register, but are separated by a distance of $\sim$3.7 Å. However, ^{2}H-NMR and neutron diffraction studies of saturated symmetric PC in the gel phase of the bilayer indicate that the two hydrocarbon chains are more nearly in register at the bilayer center (18,19). Instead of 3.7 Å as revealed by the X-ray crystal work, a separation of only 1.8 Å is observed for the two methyl groups in the gel phase of the bilayer. Experiments with dipalmitoyl PC carried out at temperatures higher than the phase transition temperature reveal that the first segment of the *sn*-2 chain, oriented parallel to the bilayer surface, has the largest statistical weight (20), indicating that the two acyl chains are, on the time average, inequivalent even in the liquid-crystalline state. In addition, ^{2}H-NMR studies by Seelig and Seelig (16) demonstrate that the conformational inequivalence of the *sn*-1 and *sn*-2 acyl chains is independent of the fatty acid composition of the PC. It would seem reasonable to assume, therefore, that the chain inequivalence in the saturated symmetric-chain PC persists independent of the chain length of the PC. Thus, in either the gel or the liquid-crystalline phase, there is a small segment of the terminal end of the *sn*-1 fatty acyl chain which must be distorted, possibly by *trans* $\leftrightarrow$ *gauche* rotational isomerization, to fill up the space under the methyl terminus of the *sn*-2 chain in order not to leave a region of vacuum. The *apparent* length of the *sn*-1 acyl chain of the saturated symmetric phospholipid that is actually in the *trans* configuration in the gel phase must, therefore, be 1.8 Å (or 1.5 carbon-carbon bond lengths) shorter than an all-*trans* chain configuration. Thus, the displaced terminal end of the *sn*-1 acyl chain would be expected to display a high degree of isotropic motion even in the gel phase of the bilayer. Region 3 may well be the source of the *gauche* rotomers, shown by Raman spectroscopy, to be present in the gel phase of saturated PC bilayers (21-23). Because of the large amount of disorder postulated to exist within region 3, it would be expected that this region will also not directly contribute, thermodynamically, to the thermal phase transition.

Region 2

Region 2 (R2) consists of the fatty acyl chain segments of the PC which lie between regions 1 and 3. In the gel phase of the bilayer, these segments can pack in association with one another and would be expected to show the largest van der Waals' attraction and the smallest volume/hydrocarbon unit of the three regions. The methylene carbon-carbon bonds within region 2 would also be expected to be predominantly in an all-*trans* configuration in the gel phase of the bilayer. It can be assumed, therefore, that region 2 will make the most significant direct contribution to the thermal phase transition of the bilayer.

Since the sizes of regions 1 and 3 remain constant, a decrease in acyl chain length will decrease the size of region 2. Thus, if region 2 were to undergo the gel $\leftrightarrow$ liquid crystalline phase transition independent of any interactions with region 1 or region 3, the thermodynamic magnitude (ΔH, ΔS, ΔV) of the transition would be expected to be a linear function of the acyl chain length. However, Figure 2 reveals that the transition entropy shows a progressively negative deviation from linearity with decreasing chain length. This observation can be interpreted in the following manner. The thermodynamic magnitude of the thermal phase transition is largely determined by the ability of the carbon units within region 2 to adopt an all-*trans* packing configuration in the bilayer gel phase which maximizes van der Waals contacts between chains. Any influence which acts to disrupt this optimal packing will decrease the thermodynamic magnitude of the phase transition. We propose here that the conformationally restricted motion of region 1 and rotomeric disorder within region 3 affect the phase transition indirectly by acting to

disrupt the regular packing of the acyl chains of region 2 within the gel phase of the bilayer. As the chain length of the PC is reduced, thus decreasing the relative size of region 2, the perturbing effects of regions 1 and 3 on the acyl chain packing would be expected to become more pronounced. This argument can account for the progressively negative deviation from linearity of the transition entropy as the PC acyl chain length is reduced.

Thus, to adequately explain the chain length dependent thermotropic behavior of these PC, it is necessary to consider not only the absolute chin length but also the relative distribution of the acyl chain carbons among the three structural regions just discussed. For this purpose we define the perturbation parameter, P, as: $P = (R_1+R_3)/R_2$. Region 1 consists of the carbonyl and α carbon of each acyl chain or 2 carbon-carbon bond lengths. Region 3, as discussed, is 1.5 C-C bond lengths. Thus, $R_1 + R_3 = 3.5$ C-C bond lengths. Region 2 contains the remaining carbon-carbon bonds or (2N-2) - 3.5 C-C bonds. The perturbation parameter, expressed as a percentage, is then given as: $P = [3.5/(2N-5.5)] \times 10^2$.

PLOT OF THERMODYNAMIC PARAMETERS VS P

The thermodynamic parameters given in Table 1 can be plotted against P; Figure 3 is a typical example of such a plot. In contrast to the curvilinear function of ΔS vs fatty acyl chain length (Fig. 2), the transition entropy is observed in Figure 3 to be a linear function of P (linear correlation coefficient = 0.998). In fact, changes in all thermodynamic parameters (Table 1) show excellent linear correlation with P.

DISCUSSION

In this communication, we propose a molecular interpretation for the chain length dependent thermotropic behavior of saturated symmetric-chain PC. It is suggested that the relatively rigid bilayer interface and rotomerically disordered terminal ends of the acyl chains perturb the conformational statistics of the rest of the hydrocarbon chain, as well as the interaction between chains, thereby preventing the acyl chains from maximizing van der Waals contacts in the bilayer gel phase. This treatment views the bilayer gel phase as becoming relatively less ordered for PC of progressively shorter acyl chain length.

It is not possible to distinguish by this analysis which of the two regions, 1 or 3, is more important to the perturbation of the

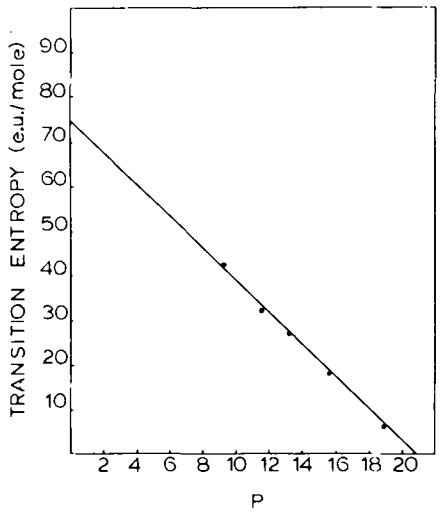

FIG. 3. Graphic demonstration of the linear correlation between the phosphatidylcholine transition entropy and the perturbation parameter, P. See text for explanation of P.

bilayer chain packing postulated here. A recent study of saturated mixed-chain PC (Mason, J.T., Huang, C., and Biltonen, R.L., submitted for publication) revealed that the thermotropic behavior of these PC is predominantly determined by the inequivalence in length of the two fatty acyl chains. The results of this study would lead us to predict that it is region 3 which is primarily responsible for the distortion of the gel phase chain packing in the saturated symmetric-chain PC, as well. However, the relative rigidity of the interface region would be expected to restrict the conformations available to the hydrocarbon methylene segments near the carbonyl end of the chain. In this way, region 1 might also make a contribution to the postulated perturbation of the hydrocarbon chain packing.

An inspection of Figure 3 and Table 1 will reveal that $\Delta H = \Delta S = \Delta V = 0$ when the perturbation parameter has a value of 20.5 ± 1.7 (SD). This observation suggests that any 1,2-diacylphosphatidylcholine with less than 11 carbons/acyl chain will not give rise to a bilayer phase transition. It is notable that Mabrey and Sturtevant (5) arrived at this same conclusion by considering the trends in transition temperature and enthalpy for saturated PC. It is obvious to state that any PC which does not form bilayers will not give rise to a bilayer phase transition. The converse statement, that any PC which does not display a bilayer phase transition will also not form bilayers, is less straightforward. It is known, however, that

diheptanoylphosphatidylcholine and dihexanoylphosphatidylcholine form micelles, not stable lamellar structures, in aqueous solution (24). These observations would suggest that the molecular interactions which are responsible for determining the magnitude of the thermal phase transition may also be involved in determining the stability of the lamellar structure.

As discussed by Phillips et al. (6), by analogy to long chain hydrocarbons, only the configurational term of the total transition entropy should be proportional to the acyl chain length in PC. The slope of Figure 3 (-3.7 e.u./mol/P) can therefore be interpreted as the decrease in configuration entropy of transition/unit increase in P. The configurational entropy of transition can reasonably be taken as a measure of the degree of rotomeric disorder along the hydrocarbon chains of the PC in the liquid-crystalline phase relative to the gel phase of the bilayer. A quantitative measure of this rotomeric disorder is the ratio of the average number of *gauche* to *trans* rotomers present along the PC fatty acyl chains (21-23). This would lead us to predict that the change in the ratio of *gauche* to *trans* rotomers which results from the thermal phase transition will decrease in strong linear correlation to an increase in P. We are currently in the process of attempting to verify this prediction experimentally.

In summary, we have stressed the importance of the bilayer interface and inequivalent conformations of the two acyl chains of the saturated PC molecule in determining the magnitude of various thermodynamic parameters associated with the gel $\longleftrightarrow$ liquid crystalline phase transition in bilayers of these PC. It would seem likely that these conformational differences will also affect other bilayer properties and should be taken into account when attempting to investigate the dynamic behavior of lipid membranes.

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