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On the main phase transition temperatures of highly asymmetric mixed-chain phosphatidylcholines

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Abstract

The highly asymmetric mixed-chain phosphatidylcholines or C(X):C(Y)PC, which can self-assemble at $T < T_m$ into the mixed interdigitated gel-state bilayer in excess water, can be divided into two groups. Group I consists of C(X):C(Y)PC with $X > Y$, and Group II consists of C(X):C(Y)PC with $X < Y$. The main phase transition temperatures (T_m) of these C(X):C(Y)PC have been analyzed in terms of two structural parameters (δ and N_f). Specifically, these structural parameters are related to the packing geometry of the lipid's acyl chains as the lipid molecules are packed either in a mixed (δ) or a hypothetically partially (N_f) interdigitated packing motif at $T < T_m$. Based on 28 and 29 T_m values of Group I and II C(X):C(Y)PC, two general equations are derived, respectively, by multiple regression analyses. These equations correlate systematically the T_m values of Group I and II phosphatidylcholines with their corresponding structural parameters. Using these two derived equations, the T_m values of a total of 92 molecular species of C(X):C(Y)PC are generated, and these calculated T_m values can be considered as the reliably predicted T_m values for highly asymmetric C(X):C(Y)PC which have $\Delta C/CL$ values within the range of 0.42–0.66.

Key words: Phase transition temperature; Mixed-chain phosphatidylcholine; Structural parameter; Mixed interdigitated packing motif

1. Introduction

Upon heating and cooling, one-component lipid bilayers comprised of saturated diacyl phospholipids exhibit, in excess water, a reversible gel to liquid-crystalline phase transition within an extremely narrow temperature range (usually < 1.0 C°). The midpoint of this narrow temperature range, commonly referred to as the main phase transition temperature abbreviated by T_m, is often recognized as a characteristic signature for that component phospholipid. One notable example is the T_m value of 41.4°C, which serves almost uniquely to characterize the best studied and fashionable lipid species of L-α-dipalmitoyl phosphatidylcholine. Most interestingly, the T_m value of a one-component phospholipid bilayer specifies the mean temperature at which all lipid molecules in that lipid bilayer are switched to undergo marked changes in inter- and intra-molecular motions as a result of temperature-induced phase transition. For instance, dipalmitoyl phosphatidylcholine molecules are essentially immobile, in terms of lateral motion, in the bilayer at $T < 41.4$°C, but highly dynamic in each of the two-dimensional planes of the two leaflets at $T > 41.4$°C with a translational diffusion coefficient on the order of 10^{-8} cm^2 s^{-1} [1]. This dynamic information could be very useful in designing experiments for assessing the effect of the physical state of lipid matrix on the activities of bilayer-spanning proteins or receptors in the reconstituted membrane system.

In the past several years, we have determined calorimetrically the T_m values of 102 molecular species of saturated diacyl phosphatidylcholines or C(X):C(Y)PC

Abbreviations: C(X):C(Y)PC, saturated 1,2-diacyl-*sn*-glycero-3-phosphocholine with X carbons in the *sn*-1 acyl chain and Y carbons in the *sn*-2 acyl chain; DSC, differential scanning calorimetry; T_m, main phase transition temperature; MW, molecular weight.

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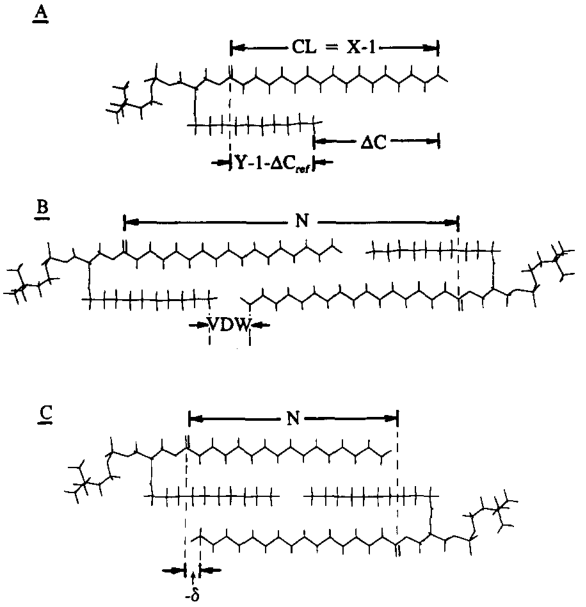

Fig. 1. Computer-generated diagrams to illustrate the various structural parameters of mixed-chain C(X):C(Y)PC. (A) A monomer of C(17):C(11)PC. CL is the effective length of the longer of the two acyl chains, and ΔC is the effective chain length difference between the two acyl chains along the long chain axis. ΔC_{ref} is the ΔC value for C(14):C(14)PC in the gel-state bilayer which is taken to be 1.5 C-C bond lengths along the long chain axis. (B) Computer drawing to illustrate a dimeric unit of C(X):C(Y)PC with a partially interdigitated packing motif. N is the hydrophobic thickness of the dimer and VDW is the van der Waals contact distance between two opposing methyl termini in the bilayer interior. (C) Computer drawing to illustrate a trans-bilayer dimer with a mixed interdigitated packing motif. N is the hydrophobic thickness of the bilayer, corresponding to the distance separating the two carbonyl oxygens of the *sn*-1 acyl chains, and δ is the difference between the length of the longer chain and the sum of the two segments of the shorter chains plus the VDW distance. The length of each segment of the short chain is defined in (A) as $Y-1-\Delta C_{\mathrm{ref}}$, corresponding to the distance from the point next to the C = O group to the chain methyl end.

[2–7]. Fifty of these molecular species have $\Delta C/CL$ values ≤ 0.41, and the other 52 molecular species have $\Delta C/CL$ values in the range of 0.43–0.63. Here, the ratio $\Delta C/CL$ is the normalized acyl chain length difference between the *sn*-1 and *sn*-2 acyl chains for C(X):C(Y)PC in the gel-state bilayer [8,9]. Hence, the magnitude of $\Delta C/CL$ reflects the acyl chain asymmetry of C(X):C(Y)PC; the larger the $\Delta C/CL$ value, the more asymmetrical is the chain length difference between the two lipid acyl chains. Specifically, ΔC is the effective chain length difference, in C-C bond lengths, between the two acyl chains (Fig. 1A), and CL is the effective length of the longer one of the two acyl chains (Fig. 1A).

Based on structural information obtained by X-ray diffraction and other calorimetric data, it has been inferred that phosphatidylcholine molecules with $\Delta C/CL$ values ≤ 0.41 tend to self-assemble, in excess water, into the partially interdigitated packing motif at $T < T_m$ [9]. In this type of gel phase packing motif, the *sn*-1 acyl chain of one phospholipid packs end to end with the *sn*-2 acyl chain of another phospholipid from the opposing leaflet (Fig. 1B). Phosphatidylcholines with $\Delta C/CL$ values in the range of 0.43–0.63, however, self-assemble at $T < T_m$, in excess water, into the mixed interdigitated packing motif [5,10], in which the longer acyl chain extends across the bilayer span and the two shorter acyl chains, each from a lipid in the opposing leaflet, meet end to end in the bilayer midplane (Fig. 1C). The switch of phospholipid packing motif from a partially interdigitated to a mixed interdigitated type at the $\Delta C/CL$ value of about 0.41–0.42 for C(X):C(Y)PC has been attributed to the change in the stabilization energy of the lipid molecule in the gel-state bilayer as a result of increasing chain asymmetry [11].

The T_m values for the 50 molecular species of C(X):C(Y)PC with $\Delta C/CL$ values ≤ 0.41 have been analyzed in terms of two structural parameters ΔC and N [6,7], where N is the thickness of the hydrocarbon core of the partially interdigitated gel-state bilayer, corresponding to the trans-bilayer distance separating the two carbonyl oxygens of the *sn*-1 acyl chains as shown in Fig. 1B. Using the statistical approach of multiple regression analysis, two simple equations have been derived in which the T_m values of C(X):C(Y)PC with $X \geq Y$ and of C(X):C(Y)PC with $X < Y$, respectively, are correlated with the linear combinations of $1/N$, $\Delta C/N$, and $\Delta C/(N + \Delta C)$ with different coefficients. These two equations have been applied to predict the T_m values for a total of 213 molecular species of C(X):C(Y)PC, each with a $\Delta C/CL$ value ≤ 0.41 [7].

The T_m values of mixed-chain C(X):C(Y)PC with $\Delta C/CL$ values in the range of 0.43–0.62 have also been analyzed previously [5]. In this early work, two structural parameters, ΔC and δ, were used to specify the T_m value. Moreover, three different equations, each containing a square term of δ and one containing, in addition, a cubic term of δ, are derived empirically to describe the T_m values of all highly asymmetric phosphatidylcholines under study [5]. Unfortunately, the derived relationships between the T_m and the structural parameters (ΔC and δ) are cumbersome; moreover, the physical meanings of those higher terms (δ^2 and δ^3) are obscure. In this communication, we have carried out additional calorimetric experiments for C(X):C(Y)PC with $\Delta C/CL$ values in the range of 0.42–0.67. These T_m values, together with those reported earlier from this laboratory [5,12], are analyzed in terms of two structural parameters (δ and N_f), leading to two simple and improved equations that can characterize generally the phase transition temperature associated with the lipid bilayer undergoing the mixed interdigitated gel to liquid-crystalline phase transition. The definitions of δ and N_f are given in a later section.

2. Materials and methods

2.1. Materials and routine procedures

Fatty acids and lysophosphatidylcholines were purchased from Sigma (St. Louis, MO) and Avanti Polar Lipids (Alabaster, AL), respectively. Silica gel 60 (mesh number: 230–400) was obtained from EM Science (Gibbstown, NJ). All chemicals and organic solvents were of reagent and spectroscopic grades, respectively. The lipid purity was checked routinely by analytical thin-layer chromatography (TLC) on silica gel 60A plate (Whatman) and the lipid spot on the TLC plate was detected by the molybdenum blue reagent [13].

Five molecular species of saturated mixed-chain phosphatidylcholine, C(13) : C(23)PC, C(21):C(14)PC, C(22):C(14)PC, C(22):C(10)PC, and C(20):C(9)PC, were semisynthesized at room temperature using the modified procedure of Mena and Djerassi [14] as described previously [2]. The synthesized lipid was subsequently purified to about 99% purity by repeated column chromatography on Silica gel 60 as reported earlier [2].

2.2. Sample preparation

Purified phosphatidylcholine was lyophilized from benzene before weighing. The lyophilized sample was then dispersed by vortexing in a NaCl (50 mM) aqueous solution containing 5 mM phosphate buffer and 1 mM EDTA at pH 7.4 to give a final lipid concentration in the range of 3.0–6.0 mM. Prior to the differential scanning calorimetric (DSC) experiment, the lipid aqueous dispersion was stored at 0°C for a minimum of 48 h. All lipid samples were scanned at least three times in the heating mode and two times in the cooling mode.

2.3. DSC measurements

DSC experiments were performed using a high-resolution Microcal calorimeter (Model MC-2) equipped with DA-2 digital interface and data acquisition utility for automatic collection (Microcal, Northampton, MA). In all measurements, a constant heating/cooling scan rate of 15 C°/h was used. Transition temperature (T_m) and transition enthalpy (ΔH) were determined from the DSC curve using the software supplied by Microcal. The reported T_m and ΔH values were the average ones taken from the second and third DSC heating scans.

3. Results and discussion

3.1. Phase transition behavior of representative C(X):C(Y)PC

The thermotropic phase behavior of many mixed-chain C(X):C(Y)PC ranging in $\Delta C/CL$ values from 0.43 to 0.64 has been well characterized, particularly those C(X):C(Y)PC with $\Delta C/CL$ values near 0.55 [2–5,10,12]. Thermotropic properties of highly asymmetric mixed-chain C(X):C(Y)PC with $\Delta C/CL$ values slightly beyond the range of 0.43–0.64, however, are less known calorimetrically. Of the five molecular species of highly asymmetric lipid studied in this work, C(13):C(23)PC and C(20):C(9)PC have $\Delta C/CL$ values of 0.415 and 0.658, respectively. Here, we show the phase transition behavior of these two lipids.

For aqueous dispersions of C(13):C(23)PC ($\Delta C/CL$ = 0.415), two discrete endothermic transitions, peaked at 34.1 and 43.9°C, are observed in the first heating scan (Fig. 2). The low temperature peak is irreversible on cooling and immediate reheating, suggesting that it corresponds to a subtransition involving the melting of a crystalline phase. The high-temperature peak observed in the first heating scan is shifted downward by about 2.6 C° upon cooling; moreover, this exothermic peak is asymmetric with a small high-temperature shoulder at 43.8°C. Upon immediate reheating, the same dispersion exhibits a single endothermic transition peaked at 43.7°C, which is observed repeatedly in all subsequent reheatings. This reproducible transition with a T_m of 43.7°C and a ΔH of 13.8 kcal/mol is thus taken as the main phase transition for the fully hydrated sample of C(13):C(23)PC.

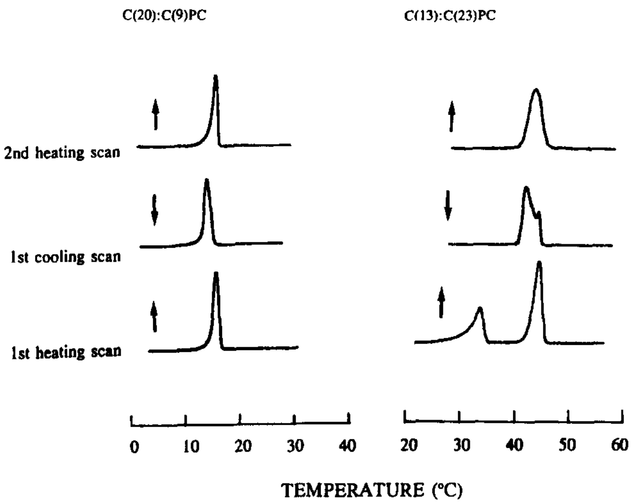

Fig. 2. The first heating, first cooling, and second heating DSC scans of C(13):C(23)PC and C(20):C(9)PC dispersions. The arrows indicate the directions of the heat flow: an upward arrow indicates an endothermic transition and a downward arrow indicates an exothermic transition. Each aqueous dispersion contains approx. 4 mM lipid in a 50 mM NaCl solution containing 5 mM phosphate buffer (pH 7.4) and 1 mM EDTA. Scan rate: 15 C°/h.

As mentioned in the Introduction, mixed-chain C(X):C(Y)PC with $\Delta C/CL$ values ≤ 0.41 tend to self-assemble, at $T < T_m$, into the partially interdigitated packing motif and those with $\Delta C/CL$ values in the range of 0.43–0.63 are packed in the mixed interdigitated gel motif. The $\Delta C/CL$ value for C(13):C(23)PC, however, lies in between 0.41 and 0.42, making the assignment of the gel-state packing motif ambiguous. Nevertheless, based on molecular mechanics calculations [11], it can be shown that the averaged overall stabilization energies of tetrameric C(13):C(23)PC packed in the partially interdigitated and mixed interdigitated motifs are essentially identical (vide post), suggesting that these two types of packing motif for the gel-state bilayer of C(13):C(23)PC can interconvert readily at $T < T_m$. Hence, we speculate that C(13):C(23)PC may self-assemble into both types of gel-state packing motif at $T < T_m$, giving rise to a mixture of two gel-state domains with similar energetic contents. The broad single transition peak observed in the second or subsequent DSC heating curve for the C(13):C(23)PC dispersion, shown in Fig. 2, may represent the envelope of two overlapped peaks, corresponding to the sum of the partially interdigitated gel → liquid-crystalline phase transition and the mixed interdigitated gel → liquid-crystalline phase transition for the fully hydrated sample of C(13):C(23)PC.

Fig. 2 also shows the successive DSC heating and cooling scans for an aqueous dispersion of C(20):C(9)PC ($\Delta C/CL = 0.658$). The heating scans are characterized by a single endothermic transition peaked at 15.7°C ($\Delta H = 10.2$ kcal/mol). A single exothermic transition peaked at 13.8°C is also exhibited by the same lipid sample upon cooling. Moreover, the single exothermic peak persists after the sample has been prolonged incubated at 0°C. This characteristic feature of down-shifting in T_m by about 2 C° on cooling suggests that the observed single phase transition for C(20):C(9)PC in the DSC heating curve cannot be attributed to the partially interdigitated → liquid-crystalline phase transition, since the T_m value of the partially interdigitated → liquid-crystalline phase transition is well known to be reversible upon cooling as exemplified by C(14):C(14)PC and C(16):C(16)PC. Instead, we assign tentatively the single transition observed in the DSC heating scan as the mixed interdigitated → liquid-crystalline phase transition. This assignment is tentative, because the aqueous dispersion of highly asymmetric lipids ($\Delta C/CL \cong 0.55$) which typically exhibits the mixed interdigitated → L_α phase transition usually shows two exothermic peaks upon cooling.

3.2. The structural parameters: ΔC, N, δ, and N_f

Based on single crystal structures of dimyristoyl phosphatidylcholine dihydrate, it is known that the diglyceride moiety of the identical-chain phospholipid exhibits a "*h*" shape geometry and the two terminal methyl groups of the *sn*-1 and *sn*-2 acyl chains are separated from each other along the chain axis by 3.70 Å or 3.00 C-C bond lengths [15]. This disparity between the *sn*-1 and *sn*-2 acyl chains persists when dimyristoyl phosphatidylcholines are packed in the gel-state bilayer with a partially interdigitated packing motif; however, the separation distance between the two chains is shortened, being about 1.5 C-C bond lengths along the bilayer normal [16]. This 1.5 C-C bond lengths difference between the two chain methyl termini for dimyristoyl phosphatidylcholine can be taken as the reference state (ΔC_{ref}) for calculating the effective chain length difference (ΔC) between two acyl chains in a mixed-chain phosphatidylcholine molecule, C(X):C(Y)PC, packed in the gel-state bilayer as follows: $\Delta C = |(X-1) - (Y - 1 - \Delta C_{\text{ref}})| = |X - Y + 1.5|$, where X and Y are the total numbers of carbon atoms in the *sn*-1 and *sn*-2 acyl chains, respectively, in a saturated mixed-chain C(X):C(Y)PC [8,9]. For instance, the value of ΔC for C(12):C(16)PC is $|12 - 16 + 1.5| = 2.5$ C-C bond lengths, and the ΔC value for its position isomer, C(16):C(12)PC, is 5.5 C-C bond lengths, indicating that the latter species is more asymmetrical in the lipid bilayer at $T < T_m$.

The segment of the *sn*-2 acyl chain from the point corresponding to the carbonyl carbon of the *sn*-1 acyl chain to the chain methyl terminus, shown in Fig. 1A, is the difference between CL and ΔC. Since CL is the length of the longer acyl chain, which is $(X-1)$ carbon-carbon bond lengths for mixed-chain lipids with $X > Y$ (Fig. 1A), and since ΔC is $(X - Y + \Delta C_{\text{ref}})$ for lipids with $X > Y$, the difference between CL and ΔC yields $(Y - 1 - \Delta C_{\text{ref}})$. This quantity is also indicated diagrammatically in Fig. 1A. The thickness of the hydrophobic core of the dimeric unit of C(X):C(Y)PC with $X > Y$ packed in the partially interdigitated motif, N, is the sum of CL, $(Y - 1 - \Delta C_{\text{ref}})$, and VDW, where VDW is the van der Waals contact distance between the two opposing terminal methyl groups in the trans-bilayer dimer as shown in Fig. 1B. If we assume the van der Waals contact distance between the two opposing methyl groups to be three C-C bond lengths along the chain, the thickness of the hydrocarbon core of the trans-bilayer dimer packed in the partially interdigitated motif can be related to the X and Y as follows: $N = (X-1) + (Y - 1 - \Delta C_{\text{ref}}) + \text{VDW} = X + Y - 0.5$. This equation is in fact also valid for mixed-chain C(X):C(Y)PC in which X is smaller than Y.

Highly asymmetric C(X):C(Y)PC ($0.42 \leq \Delta C/CL < 0.66$) have been inferred to assemble in excess water into the mixed interdigitated motif at $T < T_m$ [5,10]. The first structural parameter associated with the mixed interdigitated bilayer is δ (Fig. 1C). It is defined as the difference, within the hydrocarbon core, between the

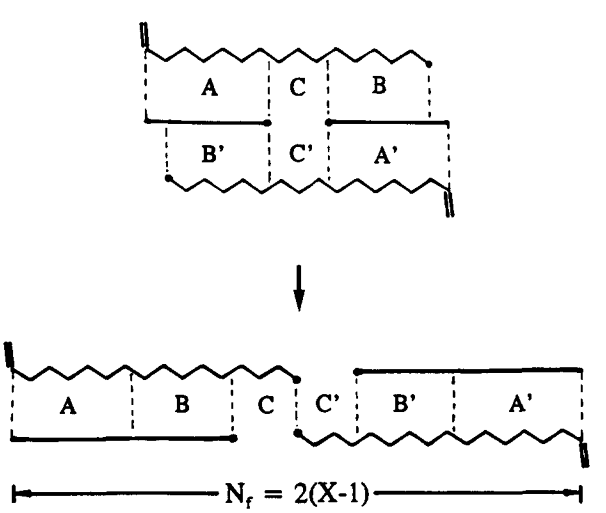

Fig. 3. The conversion of a mixed interdigitated dimer to a fictive trans-bilayer dimer with a partially interdigitated packing motif. The overall lateral chain–chain interaction and the length of the longer acyl chain are identical in these two packing models.

length of the longer chain and the sum of the two segments of the shorter chains plus the van der Waals contact distance between the chain methyl termini. For highly asymmetric C(X):C(Y)PC with $X > Y$, $\delta = (X - 1) - [2(Y - 1 - \Delta C_{\text{ref}}) + \text{VDW}] = X - 2Y - (-1 - 2\Delta C_{\text{ref}} + \text{VDW})$. When $X < Y$, $\delta = (Y - 1 - \Delta C_{\text{ref}}) - [2(X - 1) + \text{VDW}] = Y - 2X - (\Delta C_{\text{ref}} + \text{VDW} - 1)$.

A second structural parameter introduced for mixed interdigitated gel bilayer is N_f. This parameter corresponds to the thickness of the hydrocarbon core of a fictive trans-bilayer dimer with a partially interdigitated packing motif which has the same lateral chain–chain interaction as that of a trans-bilayer dimer with a mixed interdigitated packing motif. A hallmark of the trans-bilayer dimer with a mixed interdigitated packing motif is that the two shorter chains are in complementary van der Waals contacts with their two long neighboring chains (Fig. 1C), leading to an extensive lateral chain–chain interaction. We might imagine replacing the short chain by a hypothetical chain; this hypothetical chain together with the original longer chain are then rearranged into a partially interdigitated dimer. However, the overall chain–chain interaction in the fictive dimer is confined to be identical to that of the original dimer with mixed interdigitated packing motif. In Fig. 3 are drawn the actual mixed interdigitated dimer and the corresponding fictive dimer with a partially interdigitated packing motif. The overall chain–chain van der Waals contacts in these two cases are identical as represented by the A(A'), B(B'), and C(C') segments. The thickness of the hydrophobic core of the fictive dimer comprised of C(X):C(Y)PC with $X > Y$ is: $N_f = 2(X - 1)$. Similarly, one can derive the expression of $N_f = 2(Y - 1 - \Delta C_{\text{ref}})$ for highly asymmetric C(X) : C(Y)PC with $Y > X$.

The calculations of the structural parameters δ and N_f for a dimeric unit of highly asymmetric C(X):C(Y)PC depend on the values of ΔC_{ref} and VDW. For a partially interdigitated gel-state bilayer, the ΔC_{ref} value is taken to be 1.5 C-C bond lengths [8,9]. In order to be internally self-consistent, the same ΔC_{ref} value of 1.5 C-C bond lengths is used for calculating the structural parameters, δ and N_{ref}, for highly asymmetric C(X):C(Y)PC packed in the mixed interdigitated motif. The van der Waals contact distance between the two opposing methyl termini near the center of the hydrocarbon core in the mixed interdigitated bilayer (VDW) may vary somewhat, depending on the chain length of the longer acyl chain. The average value of VDW, however, can be expected to be smaller than that for the same MW but less asymmetric lipids packed in the partially interdigitated motif. This is due to the fact that the overall lateral chain–chain interaction is stronger for lipids packed in the mixed interdigitated motif [11]. One possible factor for the stronger lateral chain–chain interaction is that the steric strain or perturbation imposed by the sum of two bulky methyl groups on the neighboring straight chains is smaller due to a smaller van der Waals contact distance between the two terminal methyl groups. For a partially interdigitated gel-state bilayer, the value of VDW has been assumed to be 3 C-C bond lengths along the chain [6], corresponding to the optimal interatomic distance between two non-bonded carbon atoms [11]. The van der Waals contact distance between the two opposing methyl termini in the mixed interdigitated gel bilayer is taken here to be 3.34 Å or 2.6 C-C bond lengths; this VDW value corresponds to the closest contact packing distance between two non-bonded carbon atoms [11].

Based on the assumed values of ΔC_{ref} and VDW to be 1.5 and 2.6 C-C bond lengths, respectively, the structural parameters δ and N_f can be related to the X and Y in C(X):C(Y)PC as follows: $\delta = X - 2Y + 1.4$ and $N_f = 2(X - 1)$, if *sn*-1 is the longer chain ($X > Y$); $\delta = Y - 2X - 3.1$ and $N_f = 2(Y - 2.5)$, if *sn*-2 is the longer chain ($X < Y$).

The validity of our assumed ΔC_{ref} and VDW values is checked against the experimental data. Fig. 4 shows the plot of T_m values obtained with a homologous series of mixed-chain phosphatidylcholines as a function of $\Delta C/CL$ [4,7]. All C(X):C(Y)PC in this series have a common molecular weight identical to that of dipalmitoyl phosphatidylcholine or C(16):C(16)PC. Lipids with $X > Y$ and $X < Y$ are each seen to exhibit a biphasic curve. In the $\Delta C/CL$ range of 0.42–0.64, C(10):C(22)PC and C(21):C(11)PC give rise to the maximal T_m values in the two curves, respectively. These experimental data thus suggest that the gel-state bilayers of C(10):C(22)PC and C(21):C(11)PC are energetically more stable than those comprised of highly asym-

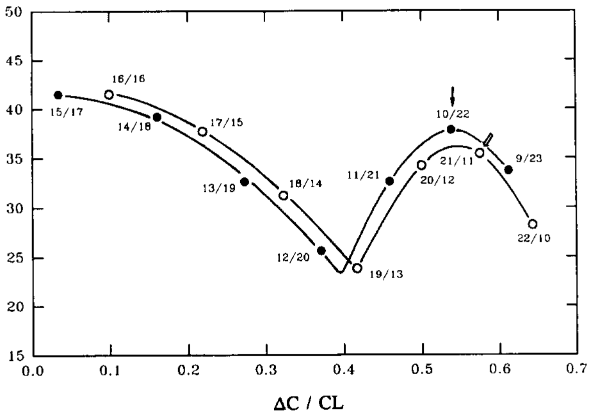

Fig. 4. Plot of the main phase transition temperature (T_m) vs. the normalized chain length difference ($\Delta C/CL$) between the two acyl chains for C(X):C(Y)PC in which $X \geq Y$ (open circles) or $X < Y$ (solid circles). All C(X):C(Y)PC used in this plot have the same MW. Data are taken from Refs. [4] and [5]. The numeric ratio, X/Y, next to each data point refers to the numbers of carbon atoms in the *sn*-1 (X) and *sn*-2 (Y) chains of the lipid species C(X):C(Y)PC.

metric lipids with the same molecular weight. Can the experimental results be consistent with the theoretical predictions based on the assumptions that $\Delta C_{\text{ref}} = 1.5$ C-C bond length and VDW = 2.6 C-C bond length? The answer to this question is given in the following paragraph.

From a structural point of view, if the total thickness of two shorter chains of highly asymmetric lipid species in a trans-bilayer dimer of mixed interdigitated motif can match perfectly with the length of the longer acyl chain at $T < T_m$, this dimeric unit will have the maximal intermolecular chain–chain contact interaction. In comparison with other highly asymmetric lipids with the same MW, this molecular species in the mixed interdigitated gel bilayer is theoretically the most stable one and hence the T_m value is also the highest. In order to show a perfect lateral matching between the long chain and the sum of two shorter chains in the mixed interdigitated gel bilayer, the δ value of the trans-bilayer dimer of C(X):C(Y)PC (Fig. 1C) must be equal to zero. We may consider first the series of C(X):C(Y)PC with $X > Y$ shown in Fig. 4; the sum of X and Y is 32 and the value of δ is $X - 2Y + 1.4$ as derived earlier. Now, let us seek out the lipid species in this series that exhibits theoretically the maximal chain–chain interaction: that is, $\delta = 0$ for the perfect lateral matching. Based on the equations of $X + Y = 32$ and $\delta = X - 2Y + 1.4 = 0$, the X and Y can be determined to be 20.87 and 11.13, respectively. The molecular species of C(X):C(Y)PC which corresponds most closely to the calculated X and Y values is thus C(21):C(11)PC. The T_m value of this calculated species of lipid is indicated by the open arrow in Fig. 4. In the case of the other series of lipids shown in Fig. 4 with $X < Y$, two simultaneous equations of $X + Y = 32$ and $\delta = Y - 2X - 3.1 = 0$ can be solved to give $X = 9.63$ and $Y = 22.37$. Closest to the calculated X and Y is the molecular species of C(10):C(22)PC, and the T_m value of the calculated lipid species of C(10):C(22)PC is indicated by the solid arrow in Fig. 4. Theoretically, these two lipid species should have the highest T_m value in their respective series of lipids due to the perfect lateral matching of their acyl chains ($\delta = 0$). The T_m values of C(21):C(11)PC and C(10):C(22)PC are indeed the maximal values of all T_m values observed experimentally in their respective curves for C(X):C(Y)PC with $\Delta C/CL$ values in the range of 0.42–0.64. The excellent agreements between the experimental data and the theoretically predicted results lend strong support to the correctness of the basic assumptions used in deriving the relationship between the structural parameters (δ and N_f) and the X and Y in C(X):C(Y)PC. The basic assumptions are twofold: $\Delta C_{\text{ref}} = 1.5$ C-C bond lengths and VDW = 2.6 C-C bond lengths.

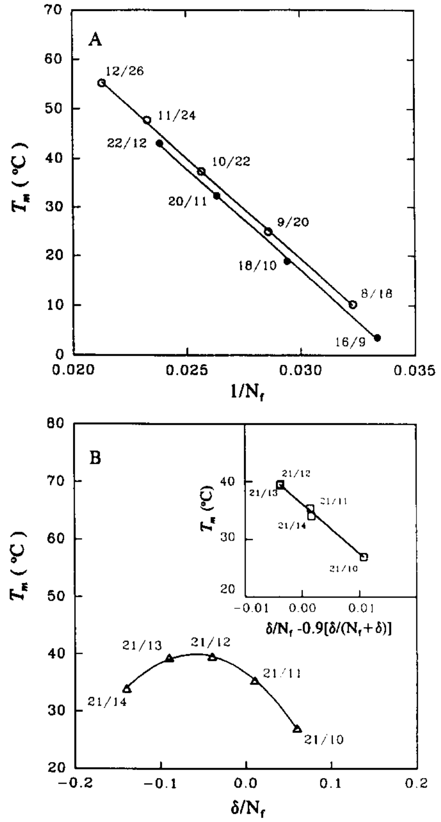

Fig. 5. Plots of the phase transition temperature vs. the structural parameters. (A) The T_m values from two series of X/Y lipids are plotted against $1/N_f$. X and Y are the total numbers of carbons in the *sn*-1 and *sn*-2 acyl chains, respectively. (B) The T_m values vs. δ/N_f. The same data are plotted against $((\delta/N_f) - 0.87\delta/(N_f + \delta))$ in the inset.

3.3. The phase transition temperature (T_m) associated with the mixed interdigitated gel → liquid-crystalline phase transition and the structural parameters δ and N_f

Fig. 5A illustrates the plot of T_m values for two series of highly asymmetric C(X):C(Y)PC ($\Delta C/CL$ = 0.53–0.57) as a function of $1/N_f$. In one series, the *sn*-1 acyl chain of C(X):C(Y)PC is longer than the *sn*-2 acyl chain, and all members share a common δ value of -0.6 carbon-carbon bond length along the chain. The negative sign of δ means that the length of the longer *sn*-1 acyl chain is smaller than the total thickness of the sum of two shorter *sn*-2 acyl chains plus the VDW distance between the two opposing methyl termini in the bilayer center based on the mixed interdigitated packing motif. A least-squares line best fitting the four T_m values of this series of C(X):C(Y)PC, shown in Fig. 5A, yields a correlation coefficient (σ) of 0.9997 and a root-mean-square error (RMSE) of 0.3918. The second series of C(X):C(Y)PC shown in Fig. 5A consists of five molecular species. Each species is characterized by a shorter *sn*-1 acyl chain and the common δ value shared by all members is -1.1 C-C bond lengths. The least-squares line best fitting the five data points in the T_m vs. $1/N_f$ plot yields σ and RMSE values of 0.9998 and 0.2968, respectively. Similar linear lines are also observed for C(X):C(Y)PC with a common $+\delta$ value.

Highly asymmetric mixed-chain phosphatidylcholines ($\Delta C/CL$ = 0.425–0.625) with a fixed longer *sn*-1 (or *sn*-2) acyl chain such as C(21):C(10)PC, C(21):C(11)PC, C(21):C(12)PC, C(21):C(13)PC, and C(21):C(14)PC have a common N_f value. When the T_m values of this series of C(21):C(Y)PC are plotted against the ratio of δ/N_f as shown in Fig. 5B, a smooth curve with the shape of a parabola is observed. This smooth curve reflects that the trend of T_m values exhibited by this series of lipids is the result of a fine delicate tuning or adjustment between the molecular weight of the lipid and the lateral mismatch of the lipid's acyl chains (δ) in the mixed interdigitated gel-state bilayer. However, if the δ/N_f value is modified by $((\delta/N_f) - 0.87$

Table 1
Calculated and experimental T_m values for fully hydrated samples of highly asymmetric C(X):C(Y)PC

Total Carbons in the *sn*-1 Acyl Chain	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
26			*37.2*	*46.3*	*53.5*	*58.3*	*60.7*	*60.2*	*56.4*									
25			*37.7*	*46.3*	*52.7*	*56.4*	*57.3*	*54.8*										
24			*37.8*	*45.8*	*51.2*	*53.5*	*52.4*											
23		*27.9*	*37.6*	*44.7*	*48.7*	*49.2*	*45.6*											
22		*27.7* **28.2**	*36.7* **37.5**	*42.6* **43.1**	*44.9* **44.6**	*43.0* **41.8**												
21		*27.0* **27.0**	*35.0* **35.4**	*39.3* **39.6**	*39.4* **39.4**	*34.3* **34.0**												
20	*15.3* **15.7**	*25.6* **26.1**	*32.2* **32.4**	*34.4* **34.2**	*31.4* **30.2**													
19	*14.1* **13.2**	*23.3* **22.7**	*27.9* **28.7**	*27.2* **27.7**														
18	*12.0* **11.0**	*19.5* **18.9**	*21.4* **21.4**	*16.6* **17.3**														
17	*8.6* **7.4**	*13.6* **13.6**	*11.6* **12.8**															
16	*3.3* **3.6**	*4.7* **4.9**	*-2.9*															
15	*-4.9*	*-8.8*																
14	*-17.5*																*51.3*	*56.4* **55.5**
13	*-37.1*														**43.7**	*48.0*	*52.9* **53.3**	*56.5*
12														*37.8* **37.6**	*44.0* **44.0**	*48.6* **48.7**	*51.9*	*54.5* **55.2**
11												*24.8* **25.7**	*33.0* **32.6**	*39.0* **38.6**	*43.4* **43.5**	*46.6* **47.7**	*49.0* **49.5**	*50.7*
10											*19.2* **19.9**	*27.1* **26.8**	*32.8* **32.7**	*37.1* **37.4**	*40.2* **40.0**	*42.4* **42.2**	*44.1* **43.1**	*45.2*
9									*1.1*	*12.0* **10.9**	*19.8* **19.6**	*25.4* **24.9**	*29.5* **29.3**	*32.5* **32.0**	*34.8* **33.7**	*36.4*		
8										*10.6* **10.2**	*16.2* **17.1**	*20.3* **21.3**	*23.4* **24.3**					

Total Carbons in the *sn*-2 Acyl Chain

The values of X and Y for C(X):C(Y)PC are specified on the vertical and horizontal axes, respectively. The experimental and calculated T_m values are given in bold-face and italic, respectively. The calculated T_m values are obtained using Eqns. 2 and 3 described in the text. The experimental T_m values of C(13):C(23)PC, C(21):C(14)PC, C(22):C(14)PC, C(22):C(10)PC, and C(20):C(9)PC were determined calorimetrically in the present study, and all other experimental T_m values were obtained previously from this laboratory [5,12].

$\delta/(N_f+\delta)$), it is exciting to note that the original parabolic curve is now substituted by a straight line as shown in the inset in Fig. 5B.

Based on the linear relationships shown in Figs. 5A and 5B, one can draw the following two conclusions. (i) The T_m values of a homologous series of highly asymmetric C(X):C(Y)PC with a common δ value are linearly related to the $1/N_f$ values with a negative slope. The smaller the $1/N_f$ value, the larger is the T_m value. (ii) For a different homologous series of highly asymmetric C(X):C(Y)PC characterized by a common N_f value, the T_m values can be related in a linear way to the function of $((\delta/N_f)-K\delta/(N_f+\delta))$, where K is a constant. These linear relationships can be combined in establishing a more general equation as follows:

$$T_m = a_0 - a_1(1/N_f) - a_2(\delta/N_f) + a_3[\delta/(N_f+\delta)] \tag{1}$$

In this equation, the first constant, a_0, represents the extrapolated T_m value for bilayers with infinite hydrophobic thickness ($N_f \to \infty$). The second term corresponds to the contribution of the fictive thickness of the hydrocarbon core of the lipid bilayer packed in the partially interdigitated motif, and this can be considered as the major term in determining the T_m value; consequently, the coefficient a_1 is most likely to be considerably greater than other coefficients (a_0, a_2 and a_3). The third term is a mismatch term contributed by the δ value and this mismatch term is modulated by the last term, a correction term. When $\delta \ll N_f$ and δ is a fixed number, the second, third, and last term of Eqn. 1 can then by approximated by a combined $-a'_1/N_f$ term, and Eqn. 1 is thus reduced to $T_m = a_0 - a'_1(1/N_f)$. Clearly, the two linear curves shown in Fig. 5A can be represented by this reduced form of Eqn. 1. When N_f is a constant, the second term of Eqn. 1 can be combined with the first term, leading to $T_m = a'_0 - a_2((\delta/N_f) - (a_3/a_2)\delta/(N_f+\delta))$, and the linear curve shown in the inset of Fig. 5B is a representation of this special case.

The T_m values of lipid bilayers prepared from highly asymmetric mixed-chain C(X):C(Y)PC ($0.42 < \Delta C/CL < 0.66$) can be divided into two groups: Group I contains T_m values obtained from C(X):C(Y)PC with longer *sn*-1 acyl chains (or $X > Y$) and Group II from C(X):C(Y)PC with $X < Y$. A total of 57 T_m values for highly asymmetric C(X):C(Y)PC, taken from earlier published values from this laboratory [5,12] together with T_m values determined in the present work, are compiled in Table 1. Group I has 28 T_m values, being individually presented in the upper left-hand corner. Group II has 29 T_m values; they are individually boxed in the lower right-hand corner. These two sets of data and the corresponding δ and N_f values were subjected to multiple regression analysis based on Eqn. 1, yielding the following two equations:

$$T_m = 140.76 - 4181.96\,(1/N_f) - 870.35(\delta/N_f) + 760.16\delta/(N_f+\delta) \tag{2}$$

with $\sigma = 0.9985$ and RMSE = 0.6441 for highly asymmetric C(X):C(Y)PC with longer *sn*-1 acyl chains ($X > Y$).

$$T_m = 139.04 - 4062.14(1/N_f) - 551.81(\delta/N_f) + 460.62\delta/(N_f+\delta) \tag{3}$$

with $\sigma = 0.9987$ and RMSE = 0.6370 for highly asymmetric C(X):C(Y)PC with longer *sn*-2 acyl chains ($X < Y$).

Using these two derived equations, a total of 92 predicted T_m values is calculated for lipid bilayers comprised of highly asymmetric C(X):C(Y)PC ($0.42 < \Delta C/CL < 0.66$). A total of 92 of these predicted values are presented in Table 1. It is interesting to compare the predicted T_m values with the experimentally obtained T_m values. Of the 28 pairs of Group I T_m values shown in Table 1 that can be compared, only two pairs show a deviation of greater than 1.0 C° and the largest deviation, 1.2 C°, is observed for C(22):C(14)PC; this largest deviation corresponds to a relative error of 0.4% in absolute temperature. In Group II phosphatidylcholines, there are 29 pairs of T_m values that can be compared. The largest deviation between the predicted and observed T_m value, 1.1 C°, is observed for C(9):C(18)PC, corresponding to a relative error of 0.4% in absolute temperature. Clearly, the agreement between the experimental and predicted T_m values is very good in all four cases. In light of these agreements, we believe that most, if not all, of the 92 predicted T_m values are reliable to within ±1.2 C°.

3.4. Limitation of the derived equations in predicting the T_m values for C(X):C(Y)PC

Fig. 4 shows the plot of experimentally determined T_m values as a function of $\Delta C/CL$ for a homologous series of C(X):C(Y)PC with MW identical to that of C(16):C(16)PC. In fact, the T_m values of three additional series of C(X):C(Y)PC with MW identical to those of C(17):C(17)PC, C(15):C(15)PC and C(14):C(14)PC, respectively, were also determined calorimetrically [2,3]. The T_m values of Group I and II mixed-chain phospholipids in each series exhibit a biphasic function similar to those shown in Fig. 4 in the T_m vs. $\Delta C/CL$ plot [6]. These biphasic curves share a common characteristic feature: the minimal T_m value for each curve occurs within a narrow $\Delta C/CL$ range of 0.41–0.42. As stated earlier, C(X):C(Y)PC with a $\Delta C/CL < 0.41$ tend to self-assemble, at $T < T_m$, into the partially interdigitated gel-bilayer, and the T_m values of these lipids can be related quite simply to the two structural parameters, ΔC and N [6,7]. In contrast, highly asymmetric C(X):C(Y)PC ranging in $\Delta C/CL$ values from 0.43 to 0.66 are inferred to pack into the mixed interdigitated bilayer at $T < T_m$, and the T_m values of these lipids can be related to the structural parameters δ and N_f as shown by Eqn. 2 and Eqn. 3 in this study. However, there are a few C(X):C(Y)PC, such as C(10):C(18)PC, C(13):C(23)PC, C(19):C(13)PC, and C(24):C(16)PC, whose $\Delta C/CL$ values fall within the small range of 0.41–0.42. An intriguing question thus arises as to which set of the structural parameters (ΔC and N vs. δ and N_f) should be used in predicting the T_m values for these C(X):C(Y)PC.

By taking into account the steric energy of C(X):C(Y)PC packed in multiple tetramers (a simple bilayer model) obtained with molecular mechanics calculations, it has been shown recently by Li et al. [11] that multiple tetramers of C(X):C(Y)PC in the crystalline state switch their packing preference near a $\Delta C/CL$ value of 0.41 from the partially interdigitated type to the mixed interdigitated type. Specifically, for C(X):C(Y)PC with $\Delta C/CL < 0.41$ in the crystalline state, these lipids can make a larger stabilization contribution to the bilayer structure with a partially interdigitated packing motif. In contrast, C(X):C(Y)PC with $\Delta C/CL > 0.42$ make a larger stabilization contribution to the bilayer with a mixed interdigitated packing motif [11]. Moreover, it is possible to compare the averaged overall stabilization energy for each molecular species within a series of C(X):C(Y)PC with identical MW. For instance, the averaged overall stabilization energy for each member of the series of C(X):C(Y)PC with MW identical to that of C(14):C(14)PC can be calculated using C(12):C(16)PC as a reference point, since the monomer of C(12):C(16)PC has the lowest steric energy, E_m [11]. In this C(X):C(Y)PC series, a Group I lipid with $\Delta C/CL = 0.404$ can be shown to have two averaged overall stabilization energies, $((E_{\text{F-B}} + E_{\text{U-D}})/2 - 4E_m]/4$:one is -11.54 kcal/mol for multiple tetramers with a partially interdigitated packing motif, and the other is -10.37 kcal/mol for tetramers with a mixed interdigitated packing motif. Here, the various values of the energy terms, $E_{\text{F-B}}$, $E_{\text{U-D}}$ and E_m, for C(X):C(Y)PC packed in either the partially interdigitated or the mixed interdigitated motif are taken from Li et al. [11]. Specifically, $E_{\text{F-B}}$ is the steric energy for two trans-bilayer dimers packed according to the front-to-back arrangement, $E_{\text{U-D}}$ is the steric energy for two trans-bilayer dimers packed in the up-and-down arrangement, and E_m is the steric energy of the monomeric lipid species. Based on the larger negative value of -11.54 kcal/mol, this Group I lipid with $\Delta C/CL = 0.404$ is shown to be present preferably in the simple multiple tetrameric model of the bilayer at $T < T_m$ as a partially interdigitated species. The packing preference for a Group II lipid with $\Delta C/CL = 0.442$ in the same series of C(X):C(Y)PC can also be calculated. The average overall stabilization energy calculated for this Group II lipid, based on the partially interdigitated motif, is -11.41 kcal/mol, and a slightly more negative value of -11.74 kcal/mol is obtained based on the mixed interdigitated motif. Although the mixed interdigitated motif is more favorable, the actual difference in the average overall stabilization energy is indeed quite small for this Group II lipid with $\Delta C/CL = 0.442$. Moreover, the difference would be even smaller for Group II lipids with $\Delta C/CL$ values in the range of 0.41–0.42 [11].

Based on molecular mechanics calculations just described, we suggest that Group I C(X):C(Y)PC ($X > Y$) with $\Delta C/CL$ in the small range of 0.41–0.42 are preferably packed in the partially interdigitated bilayer; hence, their T_m values can be predicted using the corresponding equation derived previously [7]. Group II C(X):C(Y)PC ($X < Y$) with $\Delta C/CL$ values in the 0.41–0.42 range, however, are most likely self-assembled into the gel-state bilayers with both types of packing motif, since, according to molecular mechanics calculations, C(X):C(Y)PC with a $\Delta C/CL$ value of 0.442 has an averaged overall stabilization energy which is only slightly in favor of mixed interdigitated packing motif. Consequently, Eqn. 3 cannot be applied to predict the T_m values for these Group II C(X):C(Y)PC such as C(10):C(18)PC and C(13):C(23)PC. Similarly, the equation derived previously for the T_m value of the partially interdigitated gel → L_α phase transition [7] cannot be applied to C(10):C(18)PC and C(13):C(23)PC either. In conclusion, highly asymmetric C(X):C(Y)PC, with which the $\Delta C/CL$ value is within the range of 0.41–0.42 and $X < Y$, should be considered as a special class of lipids which most likely form two types of gel-domain at $T < T_m$.

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