Phosphatidylcholines with sn-1 Saturated and sn-2 cis-Monounsaturated Acyl Chains

THEIR MELTING BEHAVIOR AND STRUCTURES*

(Received for publication, June 5, 1995, and in revised form, July 21, 1995)

Guoquan Wang, Hai-nan Lin, Shusen Li, and Ching-hsien Huang‡

From the Department of Biochemistry, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908

Recently, we have shown by high resolution differential scanning calorimetry that the position of a cis double bond (Δ-bond) in a series of 1-stearoyl-2-octadecenoyl-phosphatidylcholines can affect the phase transition temperature (Tm) or enthalpy (ΔH) of the gel-to-liquid crystalline phase transition of this series of lipids in the following manner. The value of Tm (or ΔH) is minimal when the Δ-bond is positioned at C(11) in the sn-2 acyl chain; in addition, this value increases steadily as the Δ-bond migrates toward either end of the acyl chain, resulting in a symmetrical, inverted bell-shaped profile (Wang, Z.-q., Lin, H.-n., Li, S., and Huang, C. (1995) J. Biol. Chem. 270, 2014–2023). In this communication, we have further demonstrated the inverted bell-shaped profile of Tm using 1-arachidoyl-2-eicosenoyl-phosphatidylcholines. In addition, we have extended the lipid series of 1-stearoyl-2-octadecenoyl-phosphatidylcholines to include 1-arachidoyl-2-octadecenoyl-phosphatidylcholines and 1-behenoyl-2-octadecenoyl-phosphatidylcholine, each series with a Δ-bond at varying carbon position of 6, 7, 9, 11, 12, and 13. Calorimetric results obtained with these three series of lipids indicate that the inverted bell-shaped curve shifts toward higher temperatures in a nonuniform manner as the saturated sn-1 acyl chain length increases from 17 to 19 and then to 21 C-C bond lengths. Specifically, the Tm (or ΔH) values are nearly identical for these cis-monounsaturated lipids when their Δ-bonds are positioned at C(13). Based on the height of the rotational energy barrier obtained with molecular mechanics calculations, it is evident that the rotational flexibility of the single C-C bond adjacent to the Δ-bond in 1-stearoyl-2-octadecenoyl-phosphatidylcholine increases as the Δ-bond migrates from C(9) to C(13). The differential scanning calorimetry results obtained with the three series of lipids can thus be attributed to an increase in the rotational flexibility of the short chain segment succeeding the C(14) atom in the sn-2 octadecenoyl chain. In this communication, we also propose that in the gel-state bilayer of sn-1 saturated/sn-2 cis-monounsaturated phosphatidylcholine the entire length of the shorter segment of the sn-2 acyl chain acts as a structural perturbing element; hence, it is mainly responsible for the large lower Tm of the monoenic lipid relative to the saturated counterpart. Finally, two general equations relating Tm with the structural parameters of cis-monoenoic phosphatidylcholines are presented. These equations, formulated primarily on the assumption that the short segment of the sn-2 acyl chain acts as a perturbing element, are shown to have strong predictive power in estimating the Tm values of the gel-to-liquid crystalline phase transitions for sn-1 saturated/sn-2 cis-monounsaturated phosphatidylcholines.

Phosphatidylcholines isolated from the plasma membrane of eukaryotic cells are a structurally diverse group of phospholipids. The bewildering variety of membrane phosphatidylcholines originates from the numerous possible combinations of sn-1 and sn-2 acyl chains, most of which are derived biosynthetically from saturated and unsaturated fatty acyl-CoA, respectively. By and large, the acyl chain lengths and chemical structures of the two acyl chains in a membrane phosphatidylcholine molecule are different. A good example is 1-palmitoyl-2-arachidonoyl-phosphatidylcholine, one of the most abundant lipid species found in liver cells. In the plasma membrane, phosphatidylcholine molecules aggregate in the form of the lipid bilayer due to their amphipathic nature, thus constituting the basic structural matrix. In addition, some phosphatidylcholine molecules serve as the metabolic precursors of intrinsic signaling elements, thus conferring some regulatory properties on eukaryotic cells. Consequently, it is important and relevant to investigate the intricate relationships between the structure and properties of the lipid bilayer composed of naturally occurring phosphatidylcholines.

Although the structures of a large number of phospholipids with saturated and identical number of carbon atoms in their two acyl chains have been determined by crystallographic approaches (1), the prevalence of the single-crystal structures of naturally occurring phospholipids is still elusive. However, computer-based molecular modeling for biomolecules have been advanced rapidly in recent years. This approach offers the possibility of simulating the unknown structure of naturally occurring phospholipids based on the single-crystal structures of saturated phospholipids (2). The combination of this computational approach together with calorimetric data, for example, has provided valuable information relating the structure and melting behavior of naturally occurring phospholipids in the bilayer (3, 4).

In this communication, the thermotropic phase behavior of 26 molecular species of phosphatidylcholine with sn-1 saturated/sn-2 cis-monounsaturated acyl chains was studied by high
resolution differential scanning calorimetry (DSC). These lipids were semisynthesized in this laboratory; however, they all resemble strictly the naturally occurring monoenoic phosphatidylcholines. The structures of these cis-monoenoic phosphatidylcholines were simulated using the molecular mechanics method, specifically the MM3(92) force field (5). It is well known that fully hydrated cis-monoenoic phosphatidylcholines can exhibit calorimetrically a characteristic \( T_m \), of the gel-to-liquid crystalline phase transition; moreover, this \( T_m \) is always far below that of the saturated counterpart (6, 7). We have undertaken in this work the combined approach of DSC and MM methods in order to gain a deeper understanding of the difference in \( T_m \) between the cis-monoenoic phosphatidylcholine and the saturated counterpart. Also, we will show in this work that quantitative equations relating \( T_m \) with the structural parameters for cis-monoenoic phosphatidylcholines can be developed; these equations allow us to predict the \( T_m \) values for cis-monoenoic phosphatidylcholines in general. experimental procedures Semisynthesis of Monounsaturated Phosphatidylcholines—Isomerically pure (>-98 mol %) monounsaturated phosphatidylcholines were semisynthesized on temperature by amination of \( C(14) \), \( C(16) \), and \( C(18) \)-lysophosphatidylcholine, in dry chloroform, with cis-monounsaturated fatty acid anhydride that was prepared in situ from unsaturated fatty acid and dioctylidexylo-carbodiimide, in the presence of catalyst pyridine, according to the modified procedure of Menas and Djerassi (8) as described previously (9). All reactions were carried out under an N\(_2\) atmosphere to avoid lipid oxidation. The synthesized lipids were purified by silica gel column chromatography as described elsewhere (9). All monounsaturated fatty acids with different chain lengths and distinct position of the double bond were purchased from Sigma or Nu Chek Prep, Inc. (Elysian, MN). Lysophosphatidylcholines with various saturated acyl chain lengths were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). High Resolution DSC Measurements—All DSC studies were performed on a Microcal MC-2 microcalorimeter with a DAM-2 digital interface and data acquisition utility for automatic collection (Microcal, Inc., Northampton, MA). A constant heating scan rate of 15 \(^\circ\)C/h was generally used for the DSC experiments. The \( T_m \) and \( \Delta H \) values were taken from the DSC curves after the first heating scans, and an average value for \( T_m \) or \( \Delta H \) was reported for each sample (4).

MM Calculations—The MM3 force field (version 92), obtained from Quantum Chemistry Program Exchange, Department of Chemistry, Indiana University, was used as the software package for the MM calculations to simulate the energy-minimized structures and steric energy (\( E_s \)) for various cis-monoenoic phosphatidylcholines under study. These calculations were run on an IBM RS/6000 computer workstation as described previously (4). It should be emphasized, however, that the MM3(92) was originally developed by Allinger and co-workers for simulating the colon gives the total number of carbons (\( n \)); its carbonyl carbon is C(1) and its terminal methyl carbon is C(18). For a saturated diacyl C(20):C(18)PC, for instance, has 18 carbon atoms; its carboxyl end; the acyl chain begins at the carboxyl end. The \( \Delta H \) preceding the colon in C(\( n \)):C(\( n \):1)PC refers to a single cis carbon-carbon double bond (\( \Delta \)) at the \( n \) and then to \( C(11) \) results in a progressive decrease in the conformation of 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink motif. Then, two methylene units were added to the upper segment of the sn-2 acyl chain to form the C(3):C(4) segment. The resulting crude structure of 1-arachidonoyl-2-gondoil-phosphatidylcholine was subsequently refined by subjecting to energy minimization using Allinger’s MM3 program. In order to ensure that the minimum in the potential energy surface was practically reached, additional rounds of energy minimizations were routinely performed. The application of Allinger’s program in determining the structure of lipids and the rotational energy barrier of the C-C bond in lipid acyl chain was discussed in detail elsewhere (2). Here, it is worth pointing out the reason why 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink was chosen as the starting point for the construction of other monounsaturated lipid molecules. This is due to the excellent agreement between the computational structure obtained by MM approach and the reconstructed structure based on x-ray diffraction data detected for this particular conformation of 1-palmitoyl-2-oleoyl-phosphatidylcholine (2).

results Before we present our experimental and computational results, it is appropriate to mention first the rules of nomenclature for abbreviating diacyl phospholipids adopted in this study. For a saturated diacyl lipid species, C(\( X \))C(\( Y \))PC denotes a phosphatidylcholine (PC) molecule with X and Y carbon atoms in the sn-1 and sn-2 acyl chains, respectively; hence, the notation C(\( X \)) preceding the colon in C(\( X \))C(\( Y \))PC refers to the sn-1 acyl chain with X carbons, and the notation C(\( Y \)) succeeding the colon gives the total number of carbons (\( Y \)) in the sn-2 acyl chain. The convention for numbering the carbon atom in the acyl chain begins at the carboxyl end. The sn-2 acyl chain of a saturated diacyl C(20):C(18)PC, for instance, has 18 carbon atoms; its carboxyl carbon is C(1) and its terminal methyl carbon is C(18). For a sn-1 saturated/sn-2 monounsaturated phosphatidylcholine molecule, it is abbreviated as C(\( X \))C(\( Y \)):\( \Delta \)PC. Here, we designate the position of the cis double bond as \( \Delta \), where the superscript \( n \) refers to the lower number of the two carbon atoms linked by the double bond. For instance, the double bond at the C(9)–C(10) position in the acyl chain is designated by \( \Delta 5 \). The numerical value 1 after the colon in the notation C(\( X \)):\( \Delta \)PC refers to a single cis carbon-carbon double bond (\( \Delta \)) at the n position along the sn-2 acyl chain. For 1-arachidonyl-2-gondoil-phosphatidylcholine and 1-palmitoyl-2-oleoyl-phosphatidylcholine, they can thus be abbreviated as C(20):C(18):\( \Delta \)PC and C(16):C(18):\( \Delta \)PC, respectively.

The Effect of the \( \Delta \) Position on the Phase Transition Behavior of Bilayers Composed of 1-Eicosanoyl-2-eicosanoyl-phosphatidylcholines—Fig. 1A shows four DSC heating thermograms for lipid dispersions prepared individually from C(20):C(20):\( \Delta \)PC with \( n = 5, 8, 11, \) and 13. Each thermogram is characterized by a sharp, symmetric, and pronounced endothermic phase transition that can be assigned as the gel-to-liquid crystalline phase transition or the chain melting transition. The phase transition temperature, \( T_m \), is the temperature corresponding to the maximal peak height of the transition curve. The \( T_m \) values are distinctly different for these four isomers of C(20):C(20):\( \Delta \)PC, being 44.9, 30.7, 19.7, and 22.8 \(^\circ\)C as \( n = 5, 8, 11, \) and 13, respectively.

For instance, the initial crude structural model of 1-arachidoyl-2-gondoil-phosphatidylcholine was constructed as follows: four methylene units, each being linked by the trans C=C bond, were first added to the sn-1 acyl chain of 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink motif. Then, two methylene units were added to the upper segment of the sn-2 acyl chain to form the C(3):C(4) segment. The resulting crude structure of 1-arachidonoyl-2-gondoil-phosphatidylcholine was subsequently refined by subjecting to energy minimization using Allinger’s MM3 program.

In order to ensure that the minimum in the potential energy surface was practically reached, additional rounds of energy minimizations were routinely performed. The application of Allinger’s program in determining the structure of lipids and the rotational energy barrier of the C-C bond in lipid acyl chain was discussed in detail elsewhere (2). Here, it is worth pointing out the reason why 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink was chosen as the starting point for the construction of other monounsaturated lipid molecules. This is due to the excellent agreement between the computational structure obtained by MM approach and the reconstructed structure based on x-ray diffraction data detected for this particular conformation of 1-palmitoyl-2-oleoyl-phosphatidylcholine (2).

The Effect of the \( \Delta \) Position on the Phase Transition Behavior of Bilayers Composed of 1-Eicosanoyl-2-eicosanoyl-phosphatidylcholines—Fig. 1A shows four DSC heating thermograms for lipid dispersions prepared individually from C(20):C(20):\( \Delta \)PC with \( n = 5, 8, 11, \) and 13. Each thermogram is characterized by a sharp, symmetric, and pronounced endothermic phase transition that can be assigned as the gel-to-liquid crystalline phase transition or the chain melting transition. The phase transition temperature, \( T_m \), is the temperature corresponding to the maximal peak height of the transition curve. The \( T_m \) values are distinctly different for these four isomers of C(20):C(20):\( \Delta \)PC, being 44.9, 30.7, 19.7, and 22.8 \(^\circ\)C as \( n = 5, 8, 11, \) and 13, respectively.

For instance, the initial crude structural model of 1-arachidoyl-2-gondoil-phosphatidylcholine was constructed as follows: four methylene units, each being linked by the trans C=C bond, were first added to the sn-1 acyl chain of 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink motif. Then, two methylene units were added to the upper segment of the sn-2 acyl chain to form the C(3):C(4) segment. The resulting crude structure of 1-arachidonoyl-2-gondoil-phosphatidylcholine was subsequently refined by subjecting to energy minimization using Allinger’s MM3 program.

In order to ensure that the minimum in the potential energy surface was practically reached, additional rounds of energy minimizations were routinely performed. The application of Allinger’s program in determining the structure of lipids and the rotational energy barrier of the C-C bond in lipid acyl chain was discussed in detail elsewhere (2). Here, it is worth pointing out the reason why 1-palmitoyl-2-oleoyl-phosphatidylcholine with a type IIb kink was chosen as the starting point for the construction of other monounsaturated lipid molecules. This is due to the excellent agreement between the computational structure obtained by MM approach and the reconstructed structure based on x-ray diffraction data detected for this particular conformation of 1-palmitoyl-2-oleoyl-phosphatidylcholine (2).
DSC heating thermograms for aqueous dispersions of noic phosphatidylcholines with different lipid concentration: 3–6 mM. Each aqueous lipid dispersion contains 5 second DSC heating scans obtained at a constant scan rate of 15°C/h. The area under the endothermic peak, and the H values for aqueous dispersions prepared from the four isomers of C(20):C(20:1)n-2acyl chain, are listed in Table I. The change in lipid concentration: 3–6 mM phosphate buffer (pH 7.4), 1 mM EDTA, and 50 mM NaCl. B, the plot of Tm versus the Δ-position, for the series of monoenoic lipids depicted at the left.

6, 7, 9, 11, 12, and 13 (4). The transition enthalpy (ΔH) associated with the chain melting transition is calculated from the area under the endothermic peak, and the ΔH values for aqueous dispersions prepared from the four isomers of C(20):C(20:1)n-2acyl chain, are listed in Table I. The plot of Tm versus the Δ-position, for the series of monoenoic lipids depicted at the left.

Of the four heating thermograms shown in Fig. 1A, the one exhibited by the aqueous dispersion of C(20):C(20:1)n-2acyl chain, shows a single transition with the sharpest endothermic peak centered at 30.7°C and a peak width at half-height (ΔT½) of 0.3°C. On cooling, however, the exothermic transition of the same lipid sample gives rise to a peak centered at 30.7°C with a discernible shoulder at 31.1°C (DSC curve not shown). The molecular origin of the shoulder is uncertain; nevertheless, it seems that an obligatory and transient intermediate state may exist at 31.1°C for the C(20):C(20:1)n-2acyl chain bilayer as the bilayer converts, upon cooling, from the liquid-crystalline state to the gel state. One may further speculate that monoenoic lipids in the putative state of the transient intermediate are likely to have such characteristic features that the upper and lower segments of the sn-2 acyl chain separated by the Δ-bond are in the order (or gel) and disorder (or liquid-crystalline) states, respectively.

The Effect of the Chain Length of the sn-1 Acyl Chain on the Parabolic Character of the Tm versus Δ-position Curve—Thus far, we have established the parabolic Tm - Δn curves by using lipid dispersions of C(18):C(18:1)n-2acyl chain, C(20):C(20:1)n-2acyl chain, and C(22):C(18:1)n-2acyl chain. These two series of cis-monoenoic phosphatidylcholines have a common structural feature, namely, the total numbers of carbon atoms in the two acyl chains being identical. Hence, the chain length difference between the two acyl chains (ΔC) for each lipid species is the same. In order to examine the possible effect of the sn-1 acyl chain on the parabolic character of the Tm values of cis-monoenoic phosphatidylcholines with different Δ-bond position in the sn-2 acyl chain, two additional series of monoenoic phosphatidylcholines with different ΔC values were synthesized. These two series of lipids, C(20):C(20:1)n-2acyl chain and C(22):C(20:1)n-2acyl chain with n = 6, 7, 9, 11, 12, and 13, were then studied by DSC.

Fig. 2 shows the representative DSC heating thermograms for C(20):C(18:1)n-2acyl chain and C(22):C(18:1)n-2acyl chain with n = 6, 7, 9, 11, 12, and 13. Most thermograms are characterized by a highly cooperative endothermic transition, which can be readily assigned as the chain melting or the gel-to-liquid crystalline phase transition. Of those with two endotherms, the larger transition always occurs at a higher temperature, which is assigned as the gel-to-liquid crystalline phase transition. This assignment is supported by the observation that transition characteristics of this high temperature endotherm are reproducible after repeated cooling/ heating cycles, whereas those of the low temperature transition are thermal-history dependent. For example, the C(20):C(18:1)n-2acyl chain dispersion exhibits two overlapped transitions (Fig. 2). The high temperature transition peaked at 8.5°C and is reproducible upon repeated heating or cooling. The low temperature transition, however, has a peak at 7.2°C upon heating; this peak is shifted to 7.0°C upon cooling. We, therefore, assign the high temperature transition as the gel-to-liquid crystalline phase transition for fully hydrated C(20):C(18:1)n-2acyl chain with a Tm of 8.5°C. The ΔH value of C(20):C(18:1)n-2acyl chain is calculated based on the

![Table I](https://example.com/table.png)

| Lipid | Tm°C | kcal/mol | cal/mol-K | ΔS | ΔT½°C |
|-------|------|----------|------------|----|--------|
| C(20):C(20:1)6PC | 44.9 | 8.2 ± 0.5 | 25.8 ± 1.7 | 0.5 | 22.1 |
| C(20):C(20:1)7PC | 30.7 | 7.8 ± 0.4 | 25.7 ± 0.0 | 0.3 | 22.5 |
| C(20):C(20:1)8PC | 19.7 | 7.5 ± 0.3 | 25.6 ± 1.4 | 0.6 | 21.9 |
| C(20):C(20:1)9PC | 22.8 | 7.3 ± 0.5 | 24.7 ± 1.7 | 0.6 | 22.6 |
| C(18):C(18:1)6PC | 24.8 | 7.1 ± 0.5 | 23.8 ± 1.7 | 0.7 | 21.8 |
| C(18):C(18:1)7PC | 16.7 | 6.8 ± 0.4 | 23.5 ± 1.4 | 0.3 | 21.2 |
| C(18):C(18:1)8PC | 5.6 | 6.5 ± 0.3 | 23.3 ± 1.1 | 0.8 | 20.7 |
| C(18):C(18:1)9PC | 3.8 | 6.0 ± 0.7 | 20.7 ± 2.5 | 0.8 | 20.3 |
| C(18):C(18:1)10PC | 9.1 | 6.3 ± 0.3 | 22.3 ± 1.1 | 0.9 | 21.8 |
| C(18):C(18:1)11PC | 15.9 | 6.8 ± 0.4 | 23.3 ± 1.4 | 1.1 | 22.4 |
| C(18):C(18:1)12PC | 28.5 | 7.3 ± 0.4 | 24.2 ± 1.4 | 0.3 | 22.9 |
| C(18):C(18:1)13PC | 20.9 | 7.1 ± 0.5 | 24.1 ± 1.7 | 0.6 | 22.5 |
| C(18):C(18:1)14PC | 11.0 | 7.0 ± 0.3 | 24.6 ± 1.1 | 1.0 | 23.0 |
| C(20):C(18:1)6PC | 7.0/8.5 | 6.5 ± 0.5 | 23.4 ± 1.7 | 0.9 | 22.7 |
| C(20):C(18:1)7PC | 10.2 | 6.5 ± 0.5 | 22.9 ± 1.7 | 0.9 | 22.3 |
| C(20):C(18:1)8PC | 15.9 | 6.8 ± 0.5 | 23.5 ± 1.7 | 0.8 | 22.8 |
| C(22):C(18:1)6PC | 30.5 | 7.9 ± 0.5 | 26.0 ± 1.7 | 1.0 | 25.4 |
| C(22):C(18:1)7PC | 23.7 | 7.6 ± 0.6 | 25.6 ± 2.0 | 0.4 | 24.1 |
| C(22):C(18:1)8PC | 15.1 | 7.7 ± 0.4 | 25.6 ± 1.4 | 1.0 | 24.5 |
| C(22):C(18:1)9PC | 11.5 | 7.1 ± 0.4 | 25.0 ± 1.4 | 0.4 | 23.6 |
| C(22):C(18:1)10PC | 13.2 | 7.4 ± 0.5 | 25.8 ± 1.7 | 0.3 | 24.2 |
| C(22):C(18:1)11PC | 14.6/163 | 7.4 ± 0.7 | 25.6 ± 2.4 | 0.6 | 24.9 |
| C(18):C(20:1)6PC | 18.5 | 7.1 ± 0.4 | 24.3 ± 1.4 | 0.6 | 23.7 |
| C(22):C(20:1)6PC | 23.5/24.0 | 8.0 ± 0.6 | 26.9 ± 2.0 | 0.2 | 25.3 |
| C(18):C(22:1)6PC | 19.6 | 7.2 ± 0.5 | 24.6 ± 1.7 | 0.6 | 24.0 |
| C(22):C(22:1)6PC | 23.0 | 8.0 ± 0.3 | 26.4 ± 1.1 | 1.0 | 25.6 |
| C(22):C(22:1)11PC | 32.8 | 8.3 ± 0.4 | 27.1 ± 1.4 | 0.7 | 26.5 |

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Thermal behavior exhibited by a series of cis-monoenoic phosphatidylcholines with different Δ-bond positions. A, representative DSC heating thermograms for aqueous dispersions of C(20):C(20:1)n-2acyl chain with n = 5, 8, 11, and 13. The thermograms were the second DSC heating scans obtained at a constant scan rate of 15°C/h. Lipid concentration: 3–6 mM. Each aqueous lipid dispersion contains 5 mM phosphate buffer (pH 7.4), 1 mM EDTA, and 50 mM NaCl. B, the plot of Tm versus the Δ-bond position, for the series of monoenoic lipids depicted at the left.
D

equation of to 22, respectively. In each of these least-squares analyses, an
as the point corresponding to the minimal heating curve. The values of thermodynamic parameters (\(T_m\)) overlapped peak area of the two transitions shown in the DSC heating scans.

The \(T_m\) values of the aqueous dispersions of \(C(20):C(18:1)\)PC and \(C(22):C(18:1)\)PC with \(n = 6, 7, 9, 11, 12,\) and 13. Each aqueous sample contains 3–6 mM lipid, 5 mM phosphate buffer (pH 7.4), 1 mM EDTA, and 50 mM NaCl. Scan rate: 15 °C/h. All thermograms were obtained from the second or third DSC heating scans.

The \(T_m\) values of the aqueous dispersions of \(C(20):C(18:1)\)PC and \(C(22):C(18:1)\)PC have been used to fit the calorimetric data to show how the structural variable values associated with the gel-to-liquid crystalline phase transition at each curve is upward shifted slightly as the sn-1 acyl chain length increases. In fact, the value of \(\Delta^\alpha\) associated with the gel-to-liquid crystalline phase transition of a lipid bilayer involves fundamentally the trans → gauche isomerizations of some of the C–C bonds in the hydrocarbon moieties of the lipid molecules in the lipid bilayer. In previous sections, we have presented calorimetric data to show how the structural variable \(n\) in the sn-2 acyl chain of a monoenoic lipid affects the chain melting behavior. Now, we consider the effects of \(Y\), or the effective sn-2 acyl chain length, and \(X\) on the phase transition behavior of lipid bilayers composed of cis-monoenoic phosphatidylcholines. Specifically, DSC heating thermograms for aqueous dispersions of nine different cis-monoenoic phosphatidylcholines with a common \(\Delta^\alpha\) position at \(C(13)\) in the sn-2 acyl chain were recorded. These nine lipid species can be grouped into three series: \(C(18):C(Y:1)\)PC, \(C(20):C(Y:1)\)PC, and \(C(22):C(Y:1)\)PC with \(Y = 18, 20,\) and 22. The \(T_m\) and \(\Delta H\) values associated with the gel-to-liquid crystalline phase tran-
n-1 acyl chain is fixed in each series.

In Fig. 4A, the plot of T_m as a function of the total number of carbon atoms in the sn-2 acyl chain (Y) is presented. Within the narrow range of Y from 18 to 22, the T_m is observed to be a linear function of Y for all three series of cis-monenoic lipids with constant values of X and n. The sn-2 acyl chain of a monoenoic lipid in the gel-state bilayer can be considered to consist of two linear segments separated by the cis double bond (vide post). As the Y value increases, the sn-2 acyl chain length of the lipid molecule in the gel-state bilayer increases; in particular, only the longer or the shorter segment of the sn-2 acyl chain increases due to the fixed position of Δ-bond at C(13). In addition, the thickness of the hydrocarbon core of the trans-bilayer dimer (N) and the effective chain length difference between the two acyl chains (ΔC) also change as the Y values increase in these cis-monenoic lipids. The quantitative definitions of N and ΔC in terms of X, Y, and n for cis-monenoic phosphatidylcholines are given in the next section; nevertheless, the data shown in Fig. 4A indicate that the net effect of the length of the shorter segment of the monounsaturated sn-2 acyl chain, the bilayer thickness, and the acyl chain length difference can result in a situation in which the T_m value of the lipid bilayer is linearly related to Y.

The same nine experimental T_m values shown in Fig. 4A are replotted in Fig. 4B as a function of the number of carbons in the sn-1 acyl chain (X). It should be noted that the increase in X from 18 to 20 and then to 22 for the three series of cis-monenoic lipids shown in Fig. 4B results in a corresponding increase of two C–C bond lengths in both ΔC and N values. Here, each curve in Fig. 4B reflects the subtle change in the net effect of the simultaneous increase in ΔC and N on T_m, which varies from one lipid series to the next.

Based on the experimental curves illustrated in Fig. 4A and B, it is evident that the T_m of the gel-to-liquid crystalline phase transition for cis-monenoic phospholipids can be influenced by X, Y, and ΔC, which, in turn, can be related to the structural parameters ΔC, N, and the length of the shorter segment of the sn-2 monounsaturated acyl chain. The unique value of T_m for bilayers of a given cis-monenoic phosphatidylcholine can thus be considered as a net result of the fine interplay of the three structural parameters.

Molecular Structures of Phosphatidylcholines with sn-1 Saturated/ sn-2 cis-Monounsaturated Acyl Chains as Simulated by the Molecular Mechanics Method—Since x-ray crystallographic structures of phosphatidylcholines with sn-1 saturated/sn-2 cis-unsaturated acyl chains do not exist, we have employed in this study the computer graphics-aided computational approach to simulate the structures of various cis-monenoic phosphatidylcholines. As described under “Materials and Methods,” this computational approach utilizes the MM method; specifically, the MM3(92) force field developed by Allinger and co-workers is used to generate the energy-minimized lipid structure and to calculate the steric energy of the energy-minimized lipid structure. The energy-minimized structure, however, represents the molecular structure of the lipid packed in the bilayer assembly in the crystalline state.

Three representative energy-minimized structures of C(20): C(18:1 Δ13)PC, C(20):C(20:1 Δ13)PC, and C(20):C(22:1 Δ13)PC are graphically illustrated in Fig. 5. Here, lipids in both the monomeric and dimeric states are oriented in the same manner with the zigzag plane of the all-trans sn-1 acyl chain lying on the paper (or x-y) plane. The sn-2 acyl chain of each phospholipid molecule is seen to contain two linear segments separated by a Δ-bond-containing sequence, g s Δ s as, where g and s are gauche (+) and skew (−) conformations, respectively. The zigzag planes of the two segments in the sn-2 acyl chain align perpendicularly to the paper plane: however, the long axes of the two segments run in parallel with the directionality of the sn-1 acyl chain.

If we take the chain length difference between the sn-1 and sn-2 acyl chains of C(20):C(20:1 Δ13)PC, shown in Fig. 5A, as ΔC_{ref}, where the subscript “ref” denotes the reference state, then the ΔC values for mixed-chain C(20):C(18:1 Δ13)PC and C(20):C(22:1 Δ13)PC can be easily identified from Fig. 5. B–D can be (ΔC_{ref} + 2) and (ΔC_{ref} – 2), respectively, in terms of C–C bond lengths along the long chain axis. In fact, the value of ΔC for a mixed-chain C(X):C(Y Δ13)PC can be generalized as follows: ΔC = X – Y + ΔC_{ref}. Furthermore, the chain length of the sn-1 acyl chain is X – 1 carbon-carbon bond lengths, and the chain length of the monounsaturated sn-2 acyl chain, in terms of C–C bond lengths, from the point corresponding to the carbonyl carbon of the sn-1 acyl chain to the methyl terminus is X – 1 – ΔC = X – 1 – (X – Y + ΔC_{ref}) = Y – 1 – ΔC_{ref}. It is evident from Fig. 5A that this linear portion of the sn-2 acyl chain can be considered to consist of two segments separated by the Δ-bond. The length of the lower segment (LS), which extends from the olefinic carbon (C(n + 1)) to the terminal methyl carbon is (Y – n – 1), and the length of the upper segment (US) is Y – 1 – ΔC_{ref} = (Y – n + 1) – n – ΔC_{ref}. Finally, let us define the thickness of the hydrocarbon core of the trans-bilayer dimer (N), which is taken to be the length separated by the two carbonyl oxygens of the sn-1 acyl chains along the long chain axis. The value of N can be related to X and Y as follows: N = (X – 1) + VDW + (Y – 1 – ΔC_{ref}} = X + Y + 1 – ΔC_{ref}, where
Melting Behavior and Structures of Monoenoic Phospholipids

Fig. 5. The energy-minimized structures of some representative cis-monoenoic phosphatidylcholines. A, a monomer of C(20):C(20:1)PC. The total numbers of carbons in the sn-1 and sn-2 acyl chains are identical; hence, the effective chain length difference between these two acyl chains projected on the long molecular axis is defined as $\Delta L_{\text{ref}}$. In this packing motif, the sn-2 acyl chain has a sequence g’s”s” around the cis double bond. In this graphics display, the zigzag planes of the two segments of the chain separated by the sequences g’s”s” are oriented perpendicularly to the zigzag plane of the sn-1 acyl chain. The most striking feature of this packing motif is the complementary van der Waals’ interactions between the all-trans sn-1 acyl chain and the two segments of the sn-2 acyl chain. The length of the upper segment starting from the point corresponding to the carbonyl oxygen position of the sn-1 acyl chain is designated as US, while the length of the lower segment starting from the defining C(n + 1) carbon is designated as LS. The relationship between US (or LS) and other structural parameters is defined in the text and is shown in A, B, the trans-bilayer dimer of C(20):C(18:1)PC. The effective chain length difference between the sn-1 and sn-2 acyl chains within the monomer is the structural parameter $\Delta C$. The distance along the long molecular axis separating the two carbonyl oxygens in the two opposing sn-1 acyl chains is the thickness of the hydrophobic core (N), another structural parameter. C, the trans-bilayer dimer of C(20):C(20:1)PC. D, the trans-bilayer dimer of C(20):C(20:1)PC. Note that the $\Delta C$ and N values vary in (B–D), although the $\Delta L$ is fixed at C(13) in the sn-2 acyl chain for all lipid species.

VDW is the van der Waals’ distance between the two opposing terminal methyl groups from the sn-1 and sn-2 acyl chains and is taken to be 3 C–C bond lengths.

For saturated identical-chain phosphatidylcholines such as C(14):C(14)PC packed in the gel-state bilayer, the sn-1 acyl chain is effectively longer than the sn-2 acyl chain along the long molecular axis. In fact, the methyl groups of sn-1 and sn-2 acyl chains within the same lipid molecule in the gel-state bilayer are separated from each other by about 1.5 C–C bond lengths (11). In the presence of a cis C–C double bond, the sn-2 acyl chain is further shortened by about 0.8 C–C bond lengths when a sequence g’s”s” is taken into consideration (2, 4). Consequently, the value of $\Delta L_{\text{ref}}$ can be reasonably assumed to be 2.3 C–C bond lengths for C(X):C(Y)PC if the value of $\Delta L_{\text{ref}}$ is taken at C(13). The various structural parameters for C(Y)PC packed in the gel-state bilayer involving the $\Delta L_{\text{ref}}$ term, as discussed in the above paragraph, can thus be expressed as follows: $\Delta L = X - Y + 2.3; US = n - 2.3$, and N = $X + Y - 1.3$. All of these terms have the unit of C–C bond lengths. The calculated values of the various structural parameters for all the cis-monoenoic lipids under study are summarized in Table II.

**DISCUSSION**

It is well known that the gel-to-liquid crystalline phase transition behavior exhibited by fully hydrated phosphatidylcholines is modulated by many internal factors, most notably the variation in the chain length and the chemical structure of the hydrocarbon chain (12). In addition, the $T_m$ and $\Delta H$ values of the main phase transition for aqueous dispersions of monoenoic phosphatidylcholines depend critically on the position of the cis carbon-carbon double bond ($\Delta^2$) in the sn-2 acyl chain (4). For example, a parabolic $T_m - \Delta^2$ curve with the minimal $T_m$ at C(11) is obtained after a single $\Delta^2$-bond is introduced into the sn-2 acyl chain at different positions in C(18):C(18)PC. This parabolic character of the $T_m - \Delta^2$ curve has been attributed primarily to the preferentially favorable interactions between the longer linear segment of the sn-2 acyl chain with the neighboring saturated sn-1 acyl chains in the gel-state bilayer (4).

In this study, the parabolic nature of the $T_m - \Delta^2$ curve observed originally for C(18):C(18)PC is confirmed by C(20):C(20)PC (Fig. 1). In addition, we have expanded the subclass of the lipid series used in the earlier work by including synthetic mixed-chain phosphatidylcholines in which the total number of carbon atoms in the sn-1 acyl chain is different from that in the sn-2 acyl chain. Our DSC results shown in Fig. 3 indicate that the $T_m$-lowering effect of the $\Delta^2$-bond in the sn-2 acyl chain is clearly influenced by the length of the sn-1 acyl chain when the $\Delta^2$-bond is located near the center of the chain. By contrast, the $T_m$-lowering effect of the $\Delta^2$-bond at C(13) is unchanged as the saturated sn-1 acyl chain increases from 18 to 22 carbon atoms (Fig. 3). The questions of exactly how the increase in the sn-1 chain length can affect the $T_m$ (or $\Delta H$) of mixed-chain monounsaturated phosphatidylcholine when the cis $\Delta^2$-bond is near the center of the sn-2 acyl chain and how the effect is abolished when the cis $\Delta^2$-bond is at C(13) are discussed in the following paragraphs.

For C(X):C(Y)PC packed in the gel-state bilayer, the thickness of the hydrocarbon core of the trans-bilayer dimer (N) and the $\Delta C$ value of the monomeric lipid increase with increasing chain length of the sn-1 acyl chain, since both N and $\Delta C$ values are each increased by two C–C bond lengths. The van der Waals’ distance between two methyl groups in the trans-bilayer dimer will interact laterally, at a T below Tm, giving rise to a more stable bilayer structure. For instance, as C(18):C(18)PC is lengthened to C(20):C(18)PC, the N and $\Delta C$ values are each increased by two C–C bond lengths. The van der Waals’ distance between two methyl groups is 4.0 Å or 3.0 C–C bond lengths; consequently, the two sn-1 acyl chains of the trans-bilayer C(20):C(18)PC are laterally overlapped by about 1.3 C–C bond lengths, which can give rise to an additional favorable van der Waals’ energy that is absent in the trans-bilayer dimer of C(18):C(18)PC with a smaller N value. As a result, between the C(20):C(18)PC and the C(18):C(18)PC bilayers, the $T_m$ and $\Delta H$ values associated with the gel-to-liquid crystalline phase transition are expected to be higher for C(20):C(18)PC. Likewise, the $T_m$ (or $\Delta H$) value of C(22):C(18)PC is also expected to be higher than that of C(20):C(18)PC, since both the N and $\Delta C$ values of the
The $T_m$ and the structural parameters of gel-state monounsaturated phosphatidylcholines with a cis $\Delta^1$-bond in the sn-2 acyl chain

The definitions of various structural parameters ($\Delta C$, US, LS, and N) are given in the text and Fig. 5. $T_m^\text{calc}$ values are obtained from Equations 2 and 3 for group I and II lipids, respectively. The difference between the experimental $T_m$ (column 2) and the calculated $T_m$ (column 8) values are listed in column 9 as $\Delta T_m$. In calculating $T_m$, C(22):C(18:1)$^\text{13}$PC is not considered, since the length of sn-1 acyl chain is shorter than of sn-2 acyl chain with a negative $\Delta C$ value, and it is thus classified as group I cis-monounsaturated lipid.

| Lipid                  | $T_m$ | $\Delta C$ | US   | LS   | N   | Group | $T_m^\text{calc}$ | $\Delta T_m$ |
|------------------------|-------|------------|------|------|-----|-------|-------------------|-------------|
| C(20):C(20:1)$^{13}$PC | 44.9  | 2.3        | 2.7  | 14.0 | 38.7| II    | 45.6             | −0.7        |
| C(20):C(20:1)$^{13}$PC | 30.7  | 2.3        | 5.7  | 11.0 | 38.7| II    | 29.9             | 0.8         |
| C(20):C(20:1)$^{13}$PC | 19.7  | 2.3        | 8.7  | 8.0  | 38.7| I     | 17.6             | 2.1         |
| C(20):C(20:1)$^{13}$PC | 22.8  | 2.3        | 10.7 | 6.0  | 38.7| I     | 22.7             | 0.1         |
| C(18):C(18:1)$^{13}$PC | 24.8  | 2.3        | 3.7  | 11.0 | 34.7| II    | 23.7             | 1.1         |
| C(18):C(18:1)$^{13}$PC | 16.7  | 2.3        | 4.7  | 10.0 | 34.7| II    | 17.9             | −1.2        |
| C(18):C(18:1)$^{13}$PC | 5.6   | 2.3        | 6.7  | 8.0  | 34.7| I     | 6.2              | −0.6        |
| C(18):C(18:1)$^{13}$PC | 3.8   | 2.3        | 8.7  | 6.0  | 34.7| I     | 4.5              | −0.7        |
| C(18):C(18:1)$^{13}$PC | 9.1   | 2.3        | 9.7  | 5.0  | 34.7| I     | 8.6              | 0.5         |
| C(18):C(18:1)$^{13}$PC | 15.9  | 2.3        | 10.7 | 4.0  | 34.7| I     | 14.8             | 1.1         |
| C(20):C(18:1)$^{13}$PC | 28.5  | 4.3        | 3.7  | 11.0 | 36.7| II    | 27.0             | 1.5         |
| C(20):C(18:1)$^{13}$PC | 20.9  | 4.3        | 4.7  | 10.0 | 36.7| II    | 21.4             | −0.6        |
| C(20):C(18:1)$^{13}$PC | 11.0  | 4.3        | 6.7  | 8.0  | 36.7| II    | 10.5             | 0.5         |
| C(20):C(18:1)$^{13}$PC | 7.26/8.5 | 4.3 | 8.7  | 6.0  | 36.7| I     | 8.5              | 0           |
| C(20):C(18:1)$^{13}$PC | 10.2  | 4.3        | 9.7  | 5.0  | 36.7| I     | 11.4             | −1.2        |
| C(20):C(18:1)$^{13}$PC | 15.9  | 4.3        | 10.7 | 4.0  | 36.7| I     | 15.9             | 0           |
| C(22):C(18:1)$^{13}$PC | 30.5  | 6.3        | 3.7  | 11.0 | 38.7| II    | 30.3             | 0.2         |
| C(22):C(18:1)$^{13}$PC | 23.7  | 6.3        | 4.7  | 10.0 | 38.7| II    | 25.1             | −1.4        |
| C(22):C(18:1)$^{13}$PC | 15.1  | 6.3        | 6.7  | 8.0  | 38.7| I     | 14.6             | 0.5         |
| C(22):C(18:1)$^{13}$PC | 13.5  | 6.3        | 8.7  | 6.0  | 38.7| I     | 13.4             | −1.1        |
| C(22):C(18:1)$^{13}$PC | 13.2  | 6.3        | 9.7  | 5.0  | 38.7| I     | 13.3             | −0.1        |
| C(22):C(18:1)$^{13}$PC | 14.6/16.3 | 6.3 | 10.7 | 4.0  | 38.7| I     | 16.1             | 0.2         |
| C(18):C(20:1)$^{13}$PC | 18.5  | 0.3        | 10.7 | 6.0  | 36.7| I     | 19.8             | −1.3        |
| C(22):C(20:1)$^{13}$PC | 23.5/24.0 | 4.3 | 10.7 | 6.0  | 40.7| I     | 24.9             | −0.9        |
| C(18):C(22:1)$^{13}$PC | 19.6  | −1.7       | 10.7 | 8.0  | 38.7| I'    | 29.6             | −0.4        |
| C(22):C(22:1)$^{13}$PC | 29.2  | 0.3        | 10.7 | 8.0  | 40.7| I     | 29.6             | 0.4         |

The Tm and the structural parameters of gel-state monounsaturated phosphatidylcholines with a cis $\Delta^1$-bond in the sn-2 acyl chain. The favorable lateral chain-chain interaction within the $\Delta C$ region in the crystalline dimeric unit of C(20):C(18:1)$^{13}$PC or C(22):C(18:1)$^{13}$PC is thus abolished at temperatures slightly below the $T_m$. The final result is that the $T_m$ (or $\Delta H$) values for C(18):C(18:1)$^{13}$PC, C(20):C(18:1)$^{13}$PC, and C(22):C(18:1)$^{13}$PC are virtually identical. It should be emphasized that the explanation given above is based on the assumption that the energy barriers for rotating the C(14)–C(15) bond in the sn-2 acyl chains of C(18):C(18:1)$^{13}$PC, C(20):C(18:1)$^{13}$PC, and C(22):C(18:1)$^{13}$PC are nearly identical at temperatures slightly below $T_m$. In fact, MM calculations show that the energy barriers for rotating the C(14)–C(15) bond in the sn-2 acyl chains for these three monoenoic lipid species are 1.88, 2.21, and 2.31 kcal/mol, respectively, when these monoenoic lipids are in the highly ordered conformations. At higher temperatures close to $T_m$, the lipid chains are more disordered near the methyl ends, and the differences in the energy barriers for rotating the C(14)–C(15) bond in the sn-2 acyl chains among these three lipid species are expected to be further reduced. Our basic assumption stated above is thus not unreasonable.

Another intriguing question about the effect of cis $\Delta^1$-bond on the phase transition behavior of fully hydrated phosphatidylcholine concerns the strikingly large reduction in Tm. For example, the $T_m$ value of the C(22):C(18)PC bilayer is 58.6°C (13), whereas that of the C(22):C(18:1)$^{13}$PC bilayer is 16.3°C (Table I). Before we offer an answer to this intriguing question, we need to first identify what structural modifications take place in the cis-monounsaturated phosphatidylcholines in comparison with the saturated counterparts and to consider the difference in $T_m$ in relation to the structural differences.
The basic structure of saturated C(X):C(Y)PC packed in the gel-state bilayer with a partially interdigitated motif can be specified by two structural parameters, ΔC and N, each of which is related to X and Y as follows (14): ΔC = |X − Y| + 1.5 and N = X + Y − 0.5. For monounsaturated C(X):C(Y):1Δ′PC packed in the same motif of gel-state bilayer, the presence of a structural modification of 1Δ′-bond in the sn-2 acyl chain increases the number of structural parameters to four (ΔC, N, US, and LS). These structural parameters are illustrated in Fig. 5.

Based on the calorimetric results obtained with 50 molecular species of saturated mixed-chain phosphatidylcholines, it has been demonstrated that the two structural parameters, N and ΔC, for a given type of saturated mixed-chain phosphatidylcholine molecules packed in the gel-state bilayer can be correlated with the Tm value of the lipid bilayer (13, 14). Specifically, the Tm, values are related to N and ΔC as follows (14):

\[ Tm = a_0 - a_1 \frac{N}{D} + a_2 (ΔC/N), \]

where the first term (a0) in the right-hand side of the equation corresponds to the extrapolated maximal Tm, value, which can be obtained with a bilayer containing an infinitely large value of N; the second term with a negative sign indicates that the Tm, value of a bilayer increases with increasing values of N; the last term, also with a negative sign, is regarded as the chain-end perturbation term, expressed as a normalized ΔC value, which shows that the perturbation becomes insignificant as N >> ΔC. More recently, this equation of Tm, has been refined to include a correction term (13); however, this modified equation does not significantly change our general interpretation of Tm. In summary, the basic idea underlying this equation is that the Tm, increases with increasing N and that the Tm, decreases with increasing ΔC for saturated mixed-chain phosphatidylcholines.

When a cis Δ-bond is incorporated into the sn-2 acyl chain of C(22):C(18)PC at C(13), the N value of the gel-state bilayer of C(22):C(18:1.13)PC, 38.7–C–C bond lengths, is reduced by 0.8 C–C bond lengths; hence, it is slightly longer than the N value (38.5) of C(22):C(17)PC. In contrast, the chain-end perturbation or ΔC value of C(22):C(18:1.13)PC is smaller than that of C(22):C(17)PC (6.3 versus 6.5). Interestingly, the Tm, value of the monoenic lipid is remarkably smaller than the saturated one (16.3 °C versus 53.2 °C). Obviously, the marked difference in Tm, exhibited by C(22):C(18:1.13)PC and C(22):C(17)PC bilayers cannot be explained completely by the structural parameters N and ΔC. Other structural parameters must, therefore, be taken into consideration when the Tm, of cis-monoenoic lipids is discussed. In fact, two additional structural parameters, US and LS, exist for C(X):C(Y):1Δ′PC packed in the gel-state bilayer, each of which can represent the length of the longer or the shorter segment of the sn-2 acyl chain, depending on the position of the Δ-bond. Here, we propose that the entire length of the shorter segment of the sn-2 acyl chain in the gel-state bilayer of C(X):C(Y):1Δ′PC acts as a structural perturbing element; hence, it is regarded as an important structural parameter that can modulate effectively the Tm, of cis-monoenoic lipids. The shorter segment is chosen because, at temperatures slightly below the Tm, it may have already, at least in part, transformed into a disordered state.

Of the four structural parameters associated with C(X):C(Y):1Δ′PC packed in the gel-state bilayer, three (N, ΔC, US or N, ΔC, LS) are independent variables. Since the shorter segment corresponds to the US when Δ′ is less than C(11) for a C(18):C(18:1.13)PC molecule and it is then switched to the LS as Δ′ ≥ C(11), we will then divide the monoenic C(X):C(Y):1Δ′PC into two general groups: group I with a longer upper segment and group II with a longer lower segment in the sn-2 acyl chain. Within each general group, lipid molecules with a longer effective sn-1 acyl chain should be treated differently from those with a longer effective monounsaturated sn-2 acyl chain. This is due to the recognition that the perturbing effect of the smaller segment of the sn-2 acyl chain is intrinsically different from that of ΔC. In this investigation, all but one of the cis-monoenoic saturated lipids under study have longer effective sn-1 acyl chains. The discussion will thus focus on those with longer effective sn-1 acyl chains.

Now, we can proceed to discuss the relationship between the Tm, and the three independent structural parameters (N, ΔC, and LS) for group I sn-1 saturated/sn-2 cis-monoenoic phosphatidylcholines. Specifically, we analyze how the individual contribution of the three structural parameters affects the Tm, value, and from these analyses we can then arrive at a quantitative equation relating Tm, to all three structural parameters.

First of all, it is worth noting that the Tm, values appearing on the right-hand side of each parabolic curve in Figs. 1B and 3 are derived from group I cis-monoenoic phosphatidylcholines that have the same N and ΔC values but distinctly different LS values. For example, C(20):C(20:1.13)PC and C(20):C(20:1.13)PC of Fig. 1B share the same common N and ΔC values of 38.7 and 2.3; however, their LS values are 8 and 6, respectively (Table II). The units for all three structural parameters are the C–C bond lengths along the long chain axis. It is important to recognize that all of the Tm, values exhibited by this group of cis-monoenoic phosphatidylcholines decrease with increases in LS (Table II); hence, the structural parameter LS may be regarded as a perturbing element for the gel-to-liquid crystalline phase transition. Next, we can identify two pairs of group I cis-monoenoic phosphatidylcholines in Table II that have the same common values of ΔC and LS but different N values; they are C(20):C(20:1.13)PC/C(18):C(18:1.13)PC and C(20):C(18:1.13)PC/C(22):C(20:1.13)PC. These two pairs of lipids can serve as examples to demonstrate that the Tm, increases with increasing N for cis-monoenoic phosphatidylcholines, provided that the other two structural parameters are held constant. In fact, this characteristic relationship is well known for saturated identical-chain phosphatidylcholines, which have the same ΔC value of 1.5 or a constant "end effect" as exemplified by the following two pairs of lipids: C(20):C(20:1PC/C(18)PC and C(16):C(16)PC/C(14):C(14)PC. The Tm, and ΔH values for the gel-to-liquid crystalline phase transition of these two pairs of identical-chain phosphatidylcholines increase progressively with increases in N (15). For cis-monoenoic phosphatidylcholines with constant values of N and LS but variable ΔC, two pairs of group I lipid species, C(20):C(18:1.13)PC/C(18:1PC and C(20:1.13)PC and C(22):C(18:1.13)PC/C(20):C(20:1.13)PC, can be found in Table II. The Tm, values exhibited by these two pairs of monounsaturated lipids have an inverse relationship with their ΔC values, indicating that the perturbing nature of the end effect increases with increasing ΔC, leading to a decrease in Tm, for the gel-to-liquid crystalline phase transition. A similar reciprocal relationship between the Tm, and the ΔC has also been observed for saturated mixed-chain phosphatidylcholines with a common value of N such as the following pairs of positional isomers: C(18):C(16)PC/C(16):C(18)PC and C(18):C(14)PC/C(14):C(18)PC (16, 17). Such a reciprocal relationship, however, applies only to position isomers that can form, in excess water, partially interdigitated bilayers at T < Tm (17).

Based on the three relationships discussed above between the Tm, of the gel-to-liquid crystalline phase transition of a given cis-monoenoic phosphatidylcholine and the underlying three structural parameters of the given molecule, a general equation can be formulated for group I sn-1 saturated/sn-2...
cis-monounsaturated phosphatidylcholines as follows.

\[ T_m = a_0 - a_1(1/L) + a_2(1/L^2) - a_3(D/C/LS) \]  

(Eq.1)

Note that the N value is always greater than LS and that the sn-1 acyl chain length is defined to be longer than the length of the sn-2 acyl chain for group I cis-monoenoic phosphatidylcholines packed in the gel-state bilayer. Consequently, as the value is 2.1°C for C(20):C(20:1)

The empirical Equation 3 contains an additional term in comparison with Equation 2, reflecting that the perturbation strength of the upper segment is less than that of the lower segment. In this equation, the term \( \Delta C/\Delta(C + US) \) is a correction term for \( \Delta C/US \), similar to an equivalent term used for saturated lipids (13). However, the basic assumption that the shorter segment of the sn-2 acyl chain acts as a structural perturbing element is retained in this equation. Moreover, the differences between the experimental and calculated \( T_m \) values, shown in Table II, for all 11 data are within 1.5°C. When Equation 3 is applied to estimate the \( T_m \) value of 1-palmitoyl-2-deoxyphosphatidylcholine, a value of –3.1°C is obtained, which is only 0.5°C smaller than the literature value of –2.6°C (6). Hence, Equation 3 can be considered as a reasonably good means to estimate the \( T_m \) value for group II cis-monoenoic phosphatidylcholines. Taken together, Equations 2 and 3 can yield \( T_m \) values of the gel-to-liquid crystal phase transition for cis-monoenoic phosphatidylcholines that agree closely with the experimental values (Table II). This, in turn, can be taken as evidence to support the postulate that the entire length of the shorter segment of the sn-2 acyl chain in a cis-monoenoic phosphatidylcholine molecule can act as a structural perturbing element in the lipid bilayer at temperatures slightly below \( T_m \). It is thus not unreasonable to suggest that the \( T_m \) (or \( \Delta H \))-lowering effect of a cis \( \Delta \)-bond is largely mediated through the shorter segment of the unsaturated acyl chain. Due to the inherent flexibility of the C=C bond adjacent to the \( \Delta \)-bond, it is most likely that the basic strength of the perturbation exerted by the shorter segment is considerably larger than that exerted by the chain-end perturbation or \( \Delta C \). A marked reduction in \( T_m \) thus becomes apparent. Moreover, the length of the shorter segment and hence the strength of the perturbation varies in an inverted V-shaped manner as a function of \( \Delta \); specifically, the length reaches the maximum as the \( \Delta \)-bond is shifted in position from either end to the geometric center of the sn-2 acyl chain. This variability is thus strikingly complementary to the inverted bell-shaped \( T_m \) profile observed in Figs. 1B and 3, leading to a coherent picture of the putative role played by the shorter segment of the unsaturated chain in modulating the phase transition behavior of the lipid bilayer.

REFERENCES

1. Pascher, I., Lundmark, M., Nyholm, P.-G., and Sundell, S. (1992) Biochim. Biophys. Acta 1113, 339–373
2. Li, S., Lin, H.-n., Wang, Z.-q., and Huang, C. (1994) Biochim. Biophys. Acta 1206, 2349–23499
3. Wang, Z., Lin, H., Li, S., and Huang, C. (1994) J. Biol. Chem. 269, 23491–23499
4. Wang, Z., Lin, H., Li, S., and Huang, C. (1995) Biochim. Biophys. Acta 1207, 2349–23499
5. Allinger, N. L., Yuh, Y. H., and Li, J.-H. (1989) J. Am. Chem. Soc. 111, 8531–8536
6. Keough, K. M. W. (1986) Biochem. Cell Biol. 64, 44–49
7. Keough, K. M. W. (1990) Biochem. Soc. Trans. 18, 835–837
8. Mean, P. L., and Djerassi, C. (1985) Chem. Phys. Lipids 37, 257–270
9. Lin, H.-n., Wang, Z.-q., and Huang, C. (1990) Biochemistry 29, 7063–7072
10. Pearson, R. H., and Pascher, I. (1979) Nature 281, 499–501
11. Boccal, G., Blditt, G., Seeigel, A., and Seeigel, J. (1979) J. Biol. Chem. 254, 693–706
12. Chapman, D. (1993) in Biomembranes: Physical Aspects (Shintzky, M., ed.) pp. 29–62, Balaban Publishers, Weinheim, Germany
13. Huang, C., Wang, Z., Lin, H., Brumbaugh, E. E., and Li, S. (1994) Biochim. Biophys. Acta 1193, 7–12
14. Huang, C., Li, S., Wang, Z.-q., and Lin, H.-n. (1993) Lipids 28, 365–370
15. Huang C. (1991) in Phospholipid-binding Antibodies (Harris, E. N., Exner, T., Hughes, G. R. V., and Asherson, R. A., eds.) pp. 3–30, CRC Press, Boca Raton, FL
16. Keough, K. M. W., and Davis, P. J. (1979) Biochemistry 18, 1453–1459
17. Huang, C., and Mason, J. T. (1986) Biochim. Biophys. Acta 864, 423–470
Phosphatidylcholines with \( sn--1 \) Saturated and \( sn--2 \) \textit{cis}-Monounsaturated Acyl Chains: THEIR MELTING BEHAVIOR AND STRUCTURES

Guoquan Wang, Hai-nan Lin, Shusen Li and Ching-hsien Huang

\textit{J. Biol. Chem.} 1995, 270:22738-22746.
doi: 10.1074/jbc.270.39.22738

Access the most updated version of this article at http://www.jbc.org/content/270/39/22738

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 15 references, 3 of which can be accessed free at http://www.jbc.org/content/270/39/22738.full.html#ref-list-1