Calorimetric Studies and Molecular Mechanics Simulations of Monounsaturated Phosphatidylethanolamine Bilayers*

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This investigation was designed to determine how information about the lipid chain melting and structural characteristics of sn-1 saturated/sn-2 monounsaturated phosphatidylethanolamines, a subclass of naturally occurring phospholipids, can be generalized among these lipid molecules. Specifically, 30 molecular species of sn-1 saturated/sn-2 unsaturated monoenoic phosphatidylethanolamines were semi-synthesized. The phase transition behavior of these monoenoic lipids in excess water was studied calorimetrically. The computer-based molecular mechanics approach was used to simulate the energy-minimized structures and to calculate energetic contributions for the diglyceride moieties of some of the 30 monoenoic lipid molecules, at T < Tm, in various aggregated states. These combined calorimetric and computational studies led to the following results and conclusions. 1) For a homologous series of monoenoic lipids with a common molecular weight and with a cis-double bond at various positions along the sn-2 acyl chain, the characteristic reduction of the main phase transition temperature (Tm) is proposed to be a result of an entropy-driven phenomenon. 2) The maximal decrease in the Tm for bilayers prepared from four series of monoenoic phosphatidylethanolamines containing a sn-2 octadecenoyl chain is shown by interpolation of calorimetric data to occur uniquely when the cis double bond is located commonly between C(10) and C(11) positions from the carboxyl end. 3) Monoenoic phosphatidylethanolamines can be divided into two groups. The Tm values obtained with bilayers of monoenoic lipids within each group can be systematically correlated with their structural parameters, leading to the derivation of two general Tm structure relationships.

The effects of cis carbon-carbon double bonds in the lipid acyl chain on the phase transition behavior of fully hydrated phospholipids have been studied extensively. For instance, the main phase transition temperature (Tm) of lipid bilayers prepared from a series of identical-chain phosphatidylcholines with 20 carbons in each chain are found to decrease progressively as a cis carbon-carbon double bond (Δ) is introduced stepwise into the sn-2 acyl chain (1). The influence of the position of the single Δ-bond in the two acyl chains of dioctadecenoyl phosphatidylcholine on the Tm is also known; the maximal decrease in Tm is observed when the Δ-bond lies between the C(9) and C(10) atoms in both acyl chains (2). However, our current knowledge about the phase transition behavior of bilayer membranes prepared from mixed-chain phospholipids lags far behind knowledge of identical-chain phospholipids (3, 4).

Phospholipids isolated from biological membranes are mainly mixed-chain lipids in which the sn-1 and sn-2 acyl chains have different numbers of carbons. Moreover, the sn-1 acyl chain is often a saturated polymethylene chain, whereas the sn-2 acyl chain is frequently an unsaturated chain with various numbers and positions of the Δ-bond. In this investigation, we have semi-synthesized 30 molecular species of sn-1 saturated/sn-2 unsaturated phosphatidylethanolamine with a single Δ-bond located at various positions along the sn-2 acyl chain. We have chosen monounsaturated phosphatidylethanolamines instead of phosphatidylcholines in this study, because the phase transition temperatures of the former are above 0 °C; hence, the phase transition behavior of these 30 lipid species can be readily studied calorimetrically. Furthermore, the molecular structures of the diglyceride moieties of these monoenoic mixed-chain phosphatidylethanolamines in the monomeric state and in the multiple tetrameric assembly have been simulated by molecular mechanics calculations, leading to the quantitative description of the structural parameters for these monoenoic lipids. Finally, the phase transition behavior of these 30 lipid species has been analyzed in terms of the structural parameters. The results show, for the first time, that the Tm values can be systematically related to the structural parameters of the underlying monoenoic lipid species.

EXPERIMENTAL PROCEDURES

Chemicals—Monounsaturated fatty acids with various chain lengths, each containing a cis double bond at different positions, were purchased from Sigma. Phospholipase D isolated from cabbage was supplied by Boehringer Mannheim. Lysophosphatidylcholines with various saturated acyl chain lengths were provided by Avanti Polar Lipids, Inc. (Alabaster, AL). 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.

Semi-synthesis of Monounsaturated Phosphatidylethanolamines—Monounsaturated phosphatidylcholines were initially semi-synthesized at room temperature by acylation of CdCl2 adducts of lysophosphatidylcholine in dry chloroform with monounsaturated fatty acid anhydride, which was prepared in situ from fatty acid and dicycloexyl carbodiimide, in the presence of catalyst 4-pyrrolidinopyridine according to the modified procedure of Mené and Djerrassi (5) as described previously (6). The in situ reaction and the resolubilization were carried out under N2 atmosphere to avoid the possible oxidation of the unsaturated fatty acid. The synthesized monounsaturated phosphatidylcholine was then converted enzymatically to the corresponding phosphatidylethanolamine by the transphosphatidylation with phospholipase D in the presence of excess amount of ethanolamine hydrochloride, at pH 5.6, according to the procedure of Comfurius and Zwaal (7) as described in detail previously (8).

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High Resolution Differential Scanning Calorimetry (DSC) Measurements—Purified monoenoic phosphatidylethanolamine was lyophilized from benzene before weighing. The lyophilized sample was then dispersed in buffered (pH 7.4) aqueous solution containing 50 mM NaCl, 1 mM EDTA, 5 mM phosphate buffer, and 0.02 mg/ml NaN₃, to give a final lipid concentration of 3.0-6.0 mM. Prior to DSC experiments, the lipid sample was frozen and mechanically homogenized following the procedure described by Koynova and Hinz (9).

DSC experiments were performed using a high resolution Microcalorimeter (model MC-2) equipped with DA-2 digital interface and data acquisition utility for automatic collection (Microcal, Northampton, MA). In all measurements, a constant scan rate of 15 °C/min was used. The transition temperature (T₁) and the transition enthalpy (ΔH) were determined, as described in Ref. 6, from the second DSC heating scans.

Molecular Mechanics (MM) Calculations—Phosphatidylethanolamine molecule is chemically composed of the polar headgroup and the nonpolar diglyceride moiety. The diglyceride moiety consists of the glycerol backbone and two (sn-1 and sn-2) acyl chains. The aim of the present study was to examine the influence of the position of a single A-bond in the sn-2 acyl chain on the structure and phase behavior of phosphatidylethanolamine bilayers. All lipids under study thus have a common headgroup of phosphoethanolamine, being different in their diglyceride moieties. Since the structures of various lipids to be modeled will be examined on a relative basis, it is justified to simplify the laborious MM calculations by considering only the diglyceride moiety of the various monounsaturated phosphatidylethanolamines. This approach is based on the reasonable assumption that interactions of the headgroup and water with the various diglyceride moieties of monounsaturated phosphatidylethanolamines are nearly identical; hence, they can be ignored when relative comparisons between lipids with the same headgroup are made. Consequently, various monounsaturated phosphatidylethanolamines without the headgroups and in the absence of water molecules are modeled exclusively in our MM calculations.

The MM2 program (version 85) supplied by Quantum Chemistry Program Exchange (Department of Chemistry, Indiana University) and originally developed by Allinger (10), was used to compute the atomic coordinates and the steric energy of the energy-minimized structure of the diglyceride moiety of the monoenoic lipid in monomers and in various aggregated states, essentially as described for the saturated phosphatidylcholine species (11). Prior to energy minimizations, the atomic coordinates of dilauroyl phosphatidylethanolamine and cholesteryl oleate derived from X-ray single crystal studies (12, 13) were used as the input data in constructing the crude structural model for C(12):C(18:1)PE. After energy-minimization, this structure served as a parent model upon which structures of other monoenoic lipids were built as described under "Results."

Using a three-dimensional periodic boundary condition, the simulated energy-minimized structure of a tetrameric lipid assembly packed in a box surrounded by 26 replicas of image boxes and its steric energy can be obtained (14). These computations were performed on a 486 platform using the MM2 program included in the software package HyperChem™ (Autodesk, Inc., Sausalito, CA) as described under "Results." In addition, the molecular representations of various energy-minimized lipid molecules illustrated in this report were computer-generated using the output of structural data files generated by MM2 programs and the visualization program of HyperChem™.

RESULTS

Phase Transition Behavior of Monounsaturated Mixed-chain Phosphatidylethanolamines with the A-Bond at Different Positions along the sn-2 Acyl Chain—The influence of the position of cis carbon-carbon double bond (A-bond) in the sn-2 acyl chain of phospholipids on the main phase transition behavior of the lipid bilayer was studied calorimetrically using four series of monounsaturated mixed-chain phosphatidylethanolamines. Members within each series share a common molecular weight and a common saturated sn-1 acyl chain. The position of the A-bond along the sn-2 acyl chain is designated by δ, where the superscript n denotes the number of carbon atom from the carboxyl end.

The first series of the mixed-chain phosphatidylethanolamine consists of C(16):C(18:1)PE, C(16):C(18:1)PE, C(16):C(18:1)PE, and C(16):C(18:1)PE, in which the common sn-1 acyl chain is that of palmitoyl. The initial heating, the first cooling, and the immediate second heating DSC scans obtained with the aqueous dispersions prepared from these four monounsaturated mixed-chain phosphatidylethanolamines are presented in Fig. 1. It is evident that the single endothermic phase transitions exhibited by aqueous dispersions of C(16):C(18:1)PE, C(16):C(18:1)PE, and C(16):C(18:1)PE are...
highly symmetrical. In addition, these endotherms are independent of the thermal history of the lipid samples; hence, they can be ascribed to the main or the gel to liquid crystalline \((L_g \rightarrow L_v)\) phase transition. The values of \(\Delta T_m\) and \(\Delta H\) associated with these main phase transitions calculated from the second DSC heating scans are summarized in Table 1. The phase transition curves of these samples obtained from cooling scans are, however, asymmetrical (Fig. 1). In fact, an overlapped doublet is discernible for the sample of C(16):C(18:1\text{A}^\beta)PE, suggesting that the monounsaturated mixed-chain phosphatidylethanolamines, in which the sn-1 acyl chains are derived from stearic, arachidic, and behenic acids, respectively, while the sn-2 acyl chains are originated from cis-octadecenoic acids with a single double bond at various positions, were also studied calorimetrically. The DSC results of their main phase transitions are summarized in Table 1. In Fig. 2, the \(T_m\) values of aqueous lipid dispersions prepared from all four series of monounsaturated mixed-chain phosphatidylethanolamines with a sn-2 octadecenoyl chain are plotted against the position of the cis double bond \((\Delta')\). Here, the \(T_m\) values are obtained from the second DSC heating thermograms. In this plot, experimental data within each lipid series display the same V-shaped profile.

### Phase Transition Behavior of Monounsaturated Mixed-chain Phosphatidylethanolamines with Variable Saturated sn-1 Acyl Chain

Fig. 2 also shows that the chain length of the sn-1 acyl chain in monoenic lipids affects the \(T_m\) value. For a given sn-2 octadecenoyl chain with a fixed cis double bond position such as C(18:1\text{A}^\beta)PE, experimental data indicate that the effect of the saturated sn-1 acyl chain on the \(T_m\) is progressively diminished as the chain length is increased (Figs. 2 and 3). We have also investigated the phase transition behavior of three additional series of monounsaturated mixed-chain phosphatidylethanolamines in which the chain lengths of the saturated sn-1 acyl chain are increased systematically, while the sn-2 acyl chains are maintained constant as \(\Delta^{11}\)-eicosenoate (20:1\text{A}^\gamma), \(\Delta^{13}\)-docosenoate (22:1\text{A}^\beta), and \(\Delta^{15}\)-tetracosenoate (24:1\text{A}^\beta), respectively. The \(T_m\), \(\Delta H\), and \(\Delta S\) values for the main

<table>
<thead>
<tr>
<th>Monoenoic lipid</th>
<th>(T_m^a) (°C)</th>
<th>(\Delta H) (kcal/mol)</th>
<th>(\Delta S) (cal mol/K)</th>
<th>(\Delta T_{m2}) (°C)</th>
<th>(T_{m2}) (°C)</th>
<th>(\Delta T_{m1}) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(16):C(18:1\text{A}^\beta)PE</td>
<td>31.4</td>
<td>6.3 ± 0.3</td>
<td>20.7 ± 1.0</td>
<td>1.1</td>
<td>31.3</td>
<td>0.1</td>
</tr>
<tr>
<td>C(18):C(18:1\text{A}^\beta)PE</td>
<td>28.1</td>
<td>6.1 ± 0.5</td>
<td>20.4 ± 1.5</td>
<td>1.0</td>
<td>26.1</td>
<td>0.0</td>
</tr>
<tr>
<td>C(16):C(18:1\text{A}^\gamma)PE</td>
<td>24.9</td>
<td>6.2 ± 0.3</td>
<td>20.8 ± 1.0</td>
<td>1.6</td>
<td>25.3</td>
<td>-0.4</td>
</tr>
<tr>
<td>C(16):C(18:1\text{A}^\alpha)PE</td>
<td>33.0</td>
<td>6.4 ± 0.3</td>
<td>20.9 ± 1.0</td>
<td>1.3</td>
<td>32.7</td>
<td>0.3</td>
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<tr>
<td>C(18):C(18:1\text{A}^\beta)PE</td>
<td>35.4</td>
<td>7.0 ± 0.4</td>
<td>22.7 ± 1.3</td>
<td>0.7</td>
<td>35.8</td>
<td>-0.4</td>
</tr>
<tr>
<td>C(18):C(18:1\text{A}^\alpha)PE</td>
<td>31.5</td>
<td>6.8 ± 0.4</td>
<td>22.3 ± 1.4</td>
<td>0.8</td>
<td>31.3</td>
<td>0.2</td>
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<tr>
<td>C(18):C(18:1\text{A}^\gamma)PE</td>
<td>29.8</td>
<td>6.9 ± 0.5</td>
<td>22.8 ± 1.7</td>
<td>1.8</td>
<td>29.5</td>
<td>0.3</td>
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<td>C(18):C(18:1\text{A}^\alpha)PE</td>
<td>32.3</td>
<td>7.1 ± 0.6</td>
<td>23.2 ± 2.0</td>
<td>0.8</td>
<td>32.7</td>
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<td>35.4</td>
<td>6.8 ± 0.5</td>
<td>22.0 ± 1.7</td>
<td>1.2</td>
<td>35.7</td>
<td>-0.3</td>
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<tr>
<td>C(20):C(18:1\text{A}^\beta)PE</td>
<td>37.9</td>
<td>7.4 ± 0.5</td>
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<td>37.8</td>
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<tr>
<td>C(20):C(18:1\text{A}^\alpha)PE</td>
<td>33.9</td>
<td>7.0 ± 0.5</td>
<td>22.8 ± 1.6</td>
<td>0.8</td>
<td>33.9</td>
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<td>C(20):C(18:1\text{A}^\gamma)PE</td>
<td>32.8</td>
<td>7.3 ± 0.3</td>
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<td>0.9</td>
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<td>C(20):C(18:1\text{A}^\beta)PE</td>
<td>37.5</td>
<td>6.9 ± 0.4</td>
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<td>1.2</td>
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<tr>
<td>C(22):C(18:1\text{A}^\beta)PE</td>
<td>38.5</td>
<td>7.6 ± 0.6</td>
<td>25.3 ± 1.9</td>
<td>1.8</td>
<td>38.3</td>
<td>0.2</td>
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<tr>
<td>C(22):C(18:1\text{A}^\alpha)PE</td>
<td>34.3</td>
<td>7.7 ± 0.5</td>
<td>25.0 ± 1.6</td>
<td>1.8</td>
<td>34.8</td>
<td>-0.5</td>
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<tr>
<td>C(22):C(18:1\text{A}^\gamma)PE</td>
<td>33.5</td>
<td>8.0 ± 0.5</td>
<td>26.1 ± 1.6</td>
<td>0.7</td>
<td>33.9</td>
<td>0.4</td>
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<tr>
<td>C(22):C(18:1\text{A}^\beta)PE</td>
<td>38.1</td>
<td>7.8 ± 0.3</td>
<td>25.1 ± 1.0</td>
<td>0.9</td>
<td>38.5</td>
<td>-0.4</td>
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<tr>
<td>C(24):C(18:1\text{A}^\beta)PE</td>
<td>35.2</td>
<td>8.1 ± 0.6</td>
<td>26.3 ± 2.0</td>
<td>1.9</td>
<td>34.9</td>
<td>0.3</td>
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<tr>
<td>C(16):C(20:1\text{A}^\beta)PE</td>
<td>30.3</td>
<td>6.8 ± 0.4</td>
<td>22.4 ± 1.3</td>
<td>0.9</td>
<td>30.8</td>
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<tr>
<td>C(18):C(20:1\text{A}^\beta)PE</td>
<td>39.5</td>
<td>7.4 ± 0.4</td>
<td>23.7 ± 1.3</td>
<td>1.1</td>
<td>38.6</td>
<td>0.9</td>
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<td>C(20):C(20:1\text{A}^\beta)PE</td>
<td>43.3</td>
<td>8.1 ± 0.5</td>
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<td>0.7</td>
<td>43.1</td>
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<td>C(22):C(20:1\text{A}^\beta)PE</td>
<td>44.6</td>
<td>8.5 ± 0.5</td>
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<td>1.7</td>
<td>45.5</td>
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<td>C(24):C(20:1\text{A}^\beta)PE</td>
<td>46.8</td>
<td>9.0 ± 0.5</td>
<td>28.1 ± 1.6</td>
<td>0.7</td>
<td>46.6</td>
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<td>C(16):C(22:1\text{A}^\beta)PE</td>
<td>35.2</td>
<td>7.2 ± 0.4</td>
<td>23.4 ± 1.3</td>
<td>0.7</td>
<td>35.4</td>
<td>0.2</td>
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<tr>
<td>C(18):C(22:1\text{A}^\beta)PE</td>
<td>42.7</td>
<td>7.6 ± 0.5</td>
<td>24.1 ± 1.6</td>
<td>1.7</td>
<td>43.3</td>
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<td>C(20):C(22:1\text{A}^\beta)PE</td>
<td>48.9</td>
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<td>48.5</td>
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<td>C(22):C(22:1\text{A}^\beta)PE</td>
<td>53.3</td>
<td>9.1 ± 0.6</td>
<td>27.9 ± 1.8</td>
<td>1.0</td>
<td>52.2</td>
<td>1.1</td>
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<td>C(18):C(24:1\text{A}^\beta)PE</td>
<td>56.9</td>
<td>8.1 ± 0.4</td>
<td>25.0 ± 1.2</td>
<td>0.8</td>
<td>50.0</td>
<td>0.9</td>
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<td>C(20):C(24:1\text{A}^\beta)PE</td>
<td>55.5</td>
<td>8.6 ± 0.4</td>
<td>25.2 ± 1.2</td>
<td>0.7</td>
<td>56.1</td>
<td>-0.6</td>
</tr>
<tr>
<td>C(22):C(24:1\text{A}^\beta)PE</td>
<td>59.7</td>
<td>9.2 ± 0.7</td>
<td>27.6 ± 2.1</td>
<td>0.7</td>
<td>60.5</td>
<td>0.8</td>
</tr>
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</table>
by monoenoic lipids. For instance, despite the large phosphatidylethanolamines are plotted against the position of the experimental data points are indicated monounsaturated sn-2 acyl chain is kept constant. However, Table I reveals some unique thermodynamic features exhibited for saturated C(X):C(Y)PE (16).

Positions along the chain length of the sn-1 acyl chain, while the chain length of the monounsaturated sn-2 acyl chain is kept constant. However, the increase is more pronounced for monoenoic lipids with shorter sn-1 acyl chains; a similar trend has also been observed for saturated C(X):C(Y)PE (16).

A closer examination of all experimental data presented in Table I reveals some unique thermodynamic features exhibited by monoenoic lipids. For instance, despite the large T_m variations, the observed ΔH values for all monoenoic lipids with a common molecular weight are, within experimental errors, largely identical. This feature is not shared by saturated phospholipids. The ΔH values for C(18):C(16)PC and C(16):C(18)PC, for example, are clearly different, being 8.0 and 9.3 kcal/mol, respectively (6). Furthermore, as the molecular weight of the monoenoic lipid increases, its ΔH value increases linearly with a positive slope of 0.25 kcal/mol/methylene unit. For identical-chain phosphatidylcholines, however, the increase in ΔH is a curvilinear function of the molecular weight (4).

MM Computations of Monomeric Species of Mixed-chain Phosphatidylethanolamines with a Single Δ-Bond at Various Positions along the sn-2 Octadecenooyl Chain—Unfortunately, no x-ray single crystal structure of any mixed-chain phospholipid molecule with an unsaturated sn-2 acyl chain is yet available. However, the structural data of an oleate chain or C(18:1Δⁿ)-chain obtained with cholesteryl oleate at 123 K in single crystals are known (18). These crystallographic data can be combined with those obtained with single crystals of diaroyal phosphatidylethanolamine to build a basic structural model for C(12):C(18:1Δⁿ)PE by computer-assisted molecular graphics (14). In this computer-graphics assembled structure, the torsion angles of the sn-2 acyl chain starting from C(3) to the chain methyl terminus are taken from the crystal data of the oleate chain of cholesteryl oleate, and all other torsion angles of the lipid molecule are those exhibited by crystalline dilauroyl phosphatidylethanolamine. Some of these torsion angles are summarized in Table II. The convention for naming the various torsion angles is illustrated in Fig. 4. This assembled structure of C(12):C(18:1Δⁿ)PE is used as a starting point for constructing the crude molecular model of other monoenoic lipids by computer graphics.

The crude structural models of monoenoic lipids constructed on the basis of the common parent model of C(12):C(18:1Δⁿ)PE were refined to relieve the molecular overcrowdings or steric strains in some moieties of the lipid molecule and, simultaneously, to increase the non-bonded interactions elsewhere in the same molecule. This structural refinement was achieved by the energy minimization approach using Allinger’s MM2 program as described previously (11, 14). Briefly, the atomic coordinates of the diglyceride backbone, all-trans sn-1 acyl chain, and the unsaturated sn-2 acyl chain of the constructed mixed-chain phosphatidylethanolamine molecule were first entered into the computer. These entries were adjusted systematically by the MM2 program to maximize the intramolecular interactions including the chain-chain non-bonded interaction via cycles of Newton-Raphson minimization technique, while the overall geometry of the lipid structure was changed slightly. This computation thus gave rise to the atomic coordinates of the refined lipid structure called the energy-minimized structure and the steric energy (Eₚ) for the refined structure. Finally, the output of the MM2 program led to the drawing of the energy-minimized structure of the lipid molecule by the computer-based molecular graphics using the software package of HyperChem™. Some of the representive energy-minimized structures of the mixed-chain monoenoic phosphatidylethanolamines and their steric energies obtained with MM calculations are presented in Fig. 5 and Table III. The torsion angles of the carbon-carbon single bonds surrounding the Δ-bond in the sn-2 acyl chain of these energy-minimized monoenoic lipids are summarized in Table II. In this table, the torsion angles of the C-C single bonds surrounding the Δ-bond are designated as δ, and δ₁, as shown in Fig. 4, where δ and δ₁ denote the torsion angles of the nth C-C single bond preceding and succeeding the Δ-bond, respectively.

Several common structural features are observed from Fig. 5 and Table II for the various energy-minimized structures of monoenoic lipids. 1) A kink is formed around the Δ-bond in the sn-2 acyl chain; consequently, the sn-2 acyl chain is transformed into two shorter segments. The long axes of these two segments and the chain axis of the all-trans sn-1 acyl chain are parallel (Fig. 5). The overall effective length of the sn-2 acyl chain is, in comparison with the corresponding saturated chain, shortened by about one carbon-carbon bond length. 2) The kink region around the Δ-bond in the sn-2 acyl chain is characterized by a common sequence motif g"s"Δs", where g" and s" are the gauche(−) and skew(+) bonds, respectively. For the various monounsaturated phosphatidylethanolamines shown in Fig. 5, the torsion angles of the various bonds in the sequence g"s"Δs" are δ, δ₁, Δ, and δ₁', respectively. This sequence has average torsion angles, calculated from data shown in Table II, of 68.5°, 120.6°, −0.2°, and 108.6°. 3) The total number of trans C-C bonds in the upper segment of the monounsaturated sn-2 acyl chain with the Δ-bond positioned at n is (n − 5). This is due to the fact that the kink sequence has one g" bond and one s" bond preceding the Δ-bond. In addition, the first two C-C bonds of the sn-2 acyl chain are s" (β₁) and g" (β₂),
Lipids with and without Periodic Boundary Conditions—The bond axis is tilted, making an average trans C-C bonds in the lower segment of a C(E1A") chain is has energy-minimized structures of various monoenoic phosphatidylethanolamines, in which the monounsaturated sn-2 acyl chain is fixed while the total number of carbons in the saturated sn-1 acyl chain varies systematically, are plotted against the total number of carbons in the sn-1 acyl chain.

 FIG. 3. The main phase transition temperatures \(T_m\) of bilayers prepared from four series of monoenoic phosphatidylethanolamines, in which the monounsaturated sn-2 acyl chain is fixed while the total number of carbons in the saturated sn-1 acyl chain varies systematically, are plotted against the total number of carbons in the sn-1 acyl chain.

The total carbon number in the sn-1 acyl chain or C(X)

**Table II**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>(\phi_1)</th>
<th>(\phi_2)</th>
<th>(\rho_1)</th>
<th>(\rho_2)</th>
<th>(\rho_3)</th>
<th>(\rho_4)</th>
<th>(\xi_1)</th>
<th>(\xi_2)</th>
<th>(\xi_3)</th>
<th>(\xi_4)</th>
<th>Angle*</th>
</tr>
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<tbody>
<tr>
<td>C(12):C(12)PE, Crystal</td>
<td>-52</td>
<td>-172</td>
<td>97</td>
<td>179</td>
<td>-119</td>
<td>65</td>
<td>70.1</td>
<td>123.1</td>
<td>0.9</td>
<td>127.6</td>
<td>173.5</td>
</tr>
<tr>
<td>Oleate chain, Crystal</td>
<td>-59.7</td>
<td>-178.4</td>
<td>82.0</td>
<td>174.8</td>
<td>-101.2</td>
<td>65.2</td>
<td>67.3</td>
<td>118.2</td>
<td>-0.1</td>
<td>108.6</td>
<td>173.5</td>
</tr>
<tr>
<td>C(16):C(18:1A1)PE</td>
<td>-59.7</td>
<td>-177.5</td>
<td>80.5</td>
<td>175.0</td>
<td>-99.4</td>
<td>65.2</td>
<td>68.7</td>
<td>119.9</td>
<td>-0.2</td>
<td>109.3</td>
<td>175.5</td>
</tr>
<tr>
<td>C(16):C(18:1A4)PE</td>
<td>-59.7</td>
<td>-177.4</td>
<td>80.3</td>
<td>174.0</td>
<td>-98.4</td>
<td>62.0</td>
<td>68.5</td>
<td>121.0</td>
<td>-0.1</td>
<td>108.9</td>
<td>176.2</td>
</tr>
<tr>
<td>C(16):C(18:1A13)PE</td>
<td>-59.6</td>
<td>-177.4</td>
<td>81.0</td>
<td>173.4</td>
<td>-100.1</td>
<td>61.6</td>
<td>68.7</td>
<td>122.3</td>
<td>-0.2</td>
<td>107.5</td>
<td>177.0</td>
</tr>
<tr>
<td>C(16):C(20:1A13)PE</td>
<td>-59.7</td>
<td>-177.4</td>
<td>80.2</td>
<td>174.0</td>
<td>-99.7</td>
<td>62.0</td>
<td>68.6</td>
<td>120.7</td>
<td>-0.1</td>
<td>109.1</td>
<td>176.5</td>
</tr>
<tr>
<td>C(16):C(20:1A9)PE</td>
<td>-59.6</td>
<td>-177.4</td>
<td>80.7</td>
<td>173.6</td>
<td>-99.7</td>
<td>61.6</td>
<td>68.9</td>
<td>121.0</td>
<td>-0.2</td>
<td>108.9</td>
<td>176.5</td>
</tr>
<tr>
<td>C(16):C(18:1A13)PE*</td>
<td>-65.9</td>
<td>-179.2</td>
<td>69.5</td>
<td>175.4</td>
<td>-86.6</td>
<td>66.5</td>
<td>72.8</td>
<td>124.7</td>
<td>-1.0</td>
<td>133.9</td>
<td>176.0</td>
</tr>
</tbody>
</table>

*a Denotes the angle in degree between the long chain axis of sn-1 acyl chain and the double bond axis.


as shown in Table II, which contribute uniquely to the sharp bend of ~90° at the C(2) position of the sn-2 acyl chain in phosphatidylethanolamine molecule. 4) The total number of trans C-C bonds in the lower segment of a C(Y:1A") chain is \(Y - n - 2\). This is due to the facts that the total number of C-C single bonds in the lower segment is \(Y - n - 1\) and that the C-C bond immediately succeeding the \(\Delta^e\)-bond, the \(\Delta^i\)-bond, has \(\phi^e\) conformation as shown in Table II. (5) The cis double bond axis is tilted, making an average 33° angle with respect to the long chain axis of the all-trans sn-1 acyl chain (Table II).

**MM Simulations of the Aggregates of Tetrameric Monoenoic Lipids with and without Periodic Boundary Conditions**—The energy-minimized structures of various monoenoic phosphatidylethanolamines shown in Fig. 5 were used to construct trans-bilayer dimers by MM simulations (11, 14). Two types of tetramers were subsequently constructed based on the energy-minimized structure of the trans-bilayer dimer obtained for each monoenoic lipid: the front-to-back (F-B) tetramer and the up-and-down (U-D) tetramer. The combination of these tetramers was aimed to mimic the orthorhombic closed-packed structure of phospholipids in the bilayer plane as described previously (11). These tetramers were subjected to energy minimization using Allinger's MM2 program, and their steric energies were recorded. The steric energies of the monomer \(E_m\), the F-B \(E_{FB}^{\phi}\) tetramer, and the U-D \(E_{UD}^{\phi}\) tetramer for four monoenoic phosphatidylethanolamines are presented in Table III. The stabilization energies of the F-B and U-D tetramers were calculated according to the monomer, \(\Delta E_{FB}^{\phi} = (E_{FB}^{\phi} - E_m^{\phi})/4\) and \(\Delta E_{UD}^{\phi} = (E_{UD}^{\phi} - 4E_m^{\phi})/4\), give rise to the overall averaged stabilization energy \(\Delta E_{FB}^{\phi} = (E_{FB}^{\phi} + \Delta E_{UD}^{\phi} - 4E_m^{\phi})/4\), which is also presented in Table III. The energy-minimized structures of the F-B and U-D tetramers for C(16):C(18:1A4)PE are illustrated in Fig. 6, A and B, respectively.

The energy-minimized F-B and U-D tetramers of four molecular species of monoenoic phosphatidylethanolamine molecules obtained by the MM2 program were also placed in rectangular boxes (33.69 x 30.48 x 56.10 Å for F-B tetramer and 50.73 x 17.25 x 56.10 Å for U-D tetramer). Three-dimensional periodic boundaries were employed to simulate an assembly of (3 x 1) boxes surrounding each of the original boxed tetramers using the MM* program of HyperChem™. Consequently, each tetramer is periodically replicated in all directions; this is analo-
The $\Delta H$ values of the lipid bilayers, prepared from the two homologous series of monoenoic lipids with which the values of $\Delta E^{\text{mm}}$ have been calculated by Allinger's MM2 program, are determined from DSC experiments (Table I). Most interestingly, a least-squares line with a linear correlation coefficient of 0.9966 and a root mean square error of 0.0910 is obtained, when the $\Delta H$ values of these two series of monoenoic lipids are plotted against their corresponding $(-\Delta E^{\text{mm}})$ values (Fig. 7). It should be mentioned that the overall averaged stabilization energy of the multiple tetramer contributed by its constituent monomer ($\Delta E^{\text{mm}}$) reflects the overall surface contact interaction among monomers in the simulated aggregated system. The larger the negative value of $\Delta E^{\text{mm}}$ is, the greater is the overall surface contact and the more stable is the aggregated system. Since the simulated aggregated tetramer is taken as a prototype model for the lipid bilayer in the gel state, the magnitude of $\Delta E^{\text{mm}}$ for a lipid species in a given homologous series thus reflects the relative stability of the gel-state bilayer composed of that lipid species. The transition enthalpy ($\Delta H$) obtained calorimetrically, on the other hand, gives a measure of the heat required to overcome the energy barrier for the lipid bilayer to undergo the gel to liquid-crystalline phase transition. The observed linear relationship between the $\Delta H$ and $-\Delta E^{\text{mm}}$ values means simply that the gel-state bilayer with a progressively increased stability requires a proportionally increased heat energy to undergo the thermally induced gel → liquid-crystalline phase transition. This, of course, is expected, provided that the energy-minimized structures of the lipid molecule, shown in Fig. 6 (A and B), used for our MM calculations of $\Delta E^{\text{mm}}$ and the basic structural model (the sum of F-B and U-D motifs) in deriving the value of $\Delta E^{\text{mm}}$ are indeed reasonable models for monoenoic lipids packed in the gel-state bilayer.

**DISCUSSION**

Early studies of the effect of the position of the A-bond on the $T_m$ value of a series of identical-chain phosphatidylcholines with a A-bond in each of the dioctadecenoyl chains led to the conclusion that the decrease in $T_m$ is maximal when both A-bonds are located between C(9) and C(10) positions in dioctadecenoyl chains (2). In the present study, we have determined the $T_m$ values of four series of monoenoic mixed-chain phosphatidylethanolamines, each having a common unsaturated sn-2 octadecenoyl chain with the A-bond mainly at different odd-carbon positions. Results indicate that the maximal decrease in $T_m$ occurs when the A-bond lies in between C(11) and C(12) atoms in the sn-2 octadecenoyl chain (Fig. 2). Based on x-ray and a battery of other spectroscopic studies, it is known that the initial segment of the sn-2 acyl chain of a phosphatidylcholine or phosphatidylethanolamine molecule in a bilayer runs parallel to the bilayer surface, and the sn-2 acyl chain is then bent sharply (−90°) at C(2) position so that the rest of the acyl chain runs parallel to the sn-1 acyl chain (12, 13, 19). Because of the sharp bend of the sn-2 acyl chain, the C(9) atoms in the sn-1 and sn-2 acyl chains are not positioned at the same level relative to the bilayer surface. The early DSC results reported by Barton and Gunstone (2) were obtained with dioctadecenoyl phosphatidylcholines having A-bonds at the same C-C bond positions in both chains; hence, the geometric positions of the A-bonds in each lipid species cannot be assigned accurately to a common integer owing to the mismatch of the two acyl chains. In the present investigation, our DSC data are derived from phosphatidylethanolamines having single A-bond in the sn-2 octadecenoyl chain only, thus allowing us to plot the $T_m$ versus the A-bond position unambiguously (Fig. 2). Consequently, the results obtained with monoenoic lipids as shown in Fig. 2 are not in complete accord with those DSC data reported earlier by Barton and Gunstone (2).
The steric energy and stabilization energy of a series of monoenoic lipids computed by MM programs with and without three-dimensional periodic boundaries

<table>
<thead>
<tr>
<th>Lipid</th>
<th>$E_\text{p}$</th>
<th>$E^{\text{up}}_\text{p}$</th>
<th>$E^{\text{up}}_\text{b}$</th>
<th>$\Delta E^{\text{up}}_\text{p}$</th>
<th>$E_\text{st}$</th>
<th>$E^{\text{up}}_\text{st}$</th>
<th>$E^{\text{up}}_\text{b}$</th>
<th>$\Delta E^{\text{up}}_\text{st}$</th>
<th>$\Delta H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(16):(C18:1A)PE</td>
<td>23.05</td>
<td>14.35</td>
<td>64.02</td>
<td>12.75</td>
<td>23.21</td>
<td>14.89</td>
<td>72.76</td>
<td>12.25</td>
<td>6.3 ± 0.3</td>
</tr>
<tr>
<td>C(16):(C18:1A)PE</td>
<td>23.03</td>
<td>16.07</td>
<td>66.29</td>
<td>17.24</td>
<td>23.19</td>
<td>16.53</td>
<td>75.14</td>
<td>11.74</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>C(16):(C18:1A)PE</td>
<td>23.26</td>
<td>17.32</td>
<td>65.45</td>
<td>19.22</td>
<td>23.41</td>
<td>17.68</td>
<td>73.35</td>
<td>12.03</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>C(16):(C18:1A)PE</td>
<td>23.45</td>
<td>17.68</td>
<td>65.57</td>
<td>13.05</td>
<td>23.60</td>
<td>17.99</td>
<td>74.57</td>
<td>12.03</td>
<td>6.4 ± 0.3</td>
</tr>
</tbody>
</table>

* $E_\text{p}$ is the steric energy calculated by MM* of HyperChem; the subscript b denotes results calculated by MM* of HyperChem with three-dimensional periodic boundaries (box: 33.69, 30.48, 56.10 Å for F-B tetramers and 50.73, 17.25, 56.10 Å for U-D tetramers).

(A) The front-to-back (F-B) packing motif:

\[ E^{\text{up}}_\text{st} = 16.08 \text{ kcal/mol} \]

(B) The up-and-down (U-D) packing motif:

\[ E^{\text{up}}_\text{st} = 66.29 \text{ kcal/mol} \]

\[ \Delta E^{\text{up}}_\text{st} = (E^{\text{up}}_\text{st}-E^{\text{up}}_\text{b})/4 = -6.46 \text{ kcal/mol} \]

Fig. 6. The energy-minimized structures of C(16):(C18:1A)PE packed as tetramers in the F-B arrangement (A) or the U-D arrangement (B). These refined aggregated structures were calculated by the MM2 (version 86) program.

![Diagram](image)

Fig. 7. The transition enthalpy ($\Delta H$) versus the negative value of the overall averaged stabilization energy of the multiple tetramer contributed by its constituent monomer ($-\Delta E^{\text{st}}_\text{p}$). The value of $\Delta H$ for each indicated lipid species was determined experimentally using high resolution DSC, and the computed value of $-\Delta E^{\text{st}}_\text{p}$ was obtained based on steric energies of the tetramer and the monomer of the lipid species as described under the "Results."

Although the detail energy-minimized structure of monoenoic lipid as a monomer differs somewhat from that of the same lipid molecule packed in the tetrameric aggregate as shown in Table III, the overall geometry of the lipid molecule and the total numbers of trans C-C bonds ($180 \pm 10^6$) appeared in the upper and lower segments of the lipid's sn-2 acyl chain remain unchanged in the two states. The energy-minimized structures of a series of C(16):(C18:1A)PE with identical molecular weight are shown in Fig. 5 (A-D). The numbers of trans C-C bonds in the upper and lower segments of the sn-2 acyl chain, or (C-C)$_{\text{upper}}$ and (C-C)$_{\text{lower}}$, for this series of monoenoic lipids can be readily calculated from the following relationships: (C-C)$_{\text{upper}} = n - 5$ and (C-C)$_{\text{lower}} = Y - n - 2$, and these numbers are also given in Fig. 5.

For a lipid bilayer to undergo the gel → liquid-crystalline phase transition, the lipid acyl chains will involve some chain packing loosening, arising mainly from trans → gauche rotational isomerizations of the C-C single bonds along the polyethylene chain. However, in an aggregated lipid assembly, trans → gauche isomerizations of C-C bonds in the lipid acyl chains are usually coupled. The 2g1 kink and 2g2 jog are two simple examples of the coupled isomerizations (3, 19). Kinks and jogs are favored, because these secondary structures do not perturb significantly the parallel packing of lipid acyl chains in the bilayer hydrocarbon interior (19). The 2g1 kink has the sequential g’tg’- and g’tg” bonds, and the 2g2 jog has the sequential g’ttg’- and g’ttg” bonds. In order to form a kink/jog along the lipid acyl chain, a segment of 3/5 consecutive trans C-C bonds is thus minimally required.

Since the main phase transition of the lipid bilayer involves basically the coupled trans → gauche isomerizations of consecutive C-C bonds in the acyl chain, it is thus relevant to identify: 1) the change in the length of consecutive trans C-C bonds in the two segments of a sn-2 acyl chain packed in the highly ordered state as the A-bond moves along the chain, and 2) the number of possible ways in which the various consecutive trans C-C bond lengths resulting from the A-bond migration can undergo coupled rotational isomerizations, at $T > T_m$, to form kinks or jogs. The sn-2 acyl chains of those identical molecular weight monoenoic lipids shown in Fig. 5 (A-D) can serve as a paradigm for such structural identification. Initially, when the A-bond lies between C(7) and C(8) atoms, the upper and lower segments of the sn-2 acyl chain have 2 and 9 consecutive trans C-C bonds, respectively, as shown in Fig. 5A. The numbers of the consecutive trans C-C bonds in the upper and lower segments of the C(18:1A) chain change successively from 2 and 9 to 4,6,8 and 7,5, respectively, as the A-bond migrates progressively from C(7) to C(9), C(11), and C(13) positions (Fig. 5, A-D).

To simplify the comparisons, let us consider first the number of possible ways in which a single g’tg’- kink can be maximally generated at $T > T_m$ in the sn-2 acyl chains. For a C(18:1A) chain, the upper segment has four consecutive trans C-C bonds. Since three consecutive trans C-C bonds are required to form a g’tg’- kink, there are only two possible ways in which a kink can be maximally formed at $T > T_m$ in this upper segment. The lower segment, however, has seven consecutive trans C-C bonds. Five possible ways can be readily identified for forming a g’tg’- kink at $T > T_m$ in this lower segment as follows; since a segment of three consecutive trans C-C bonds is required to form a kink, this leaves (7 - 3) = 4 ways to form a kink in the rest of the sequence. Thus, there are a total of 4 + 1 = 5 possible ways to form one kink in the lower segment of C(18:1A) chain. The sum of 2 and 5 gives the total number of possible ways in which a g’tg’- kink can be maximally formed in the sn-2 acyl chain, and the product of 2 and 5 gives the total number of possible ways in...
which a $g'tg'$-kink can be formed simultaneously in both segments of the same chain. Altogether, there are 17 possible ways to form a single kink and two kinks (one in each segment) in the C(18:1Δ9) chain. Similarly, the numbers of possible ways of forming a $g'tttg'$-jog in the upper and lower segments of the sn-2 (C(18:1Δ9)) chain can be identified as zero and three, respectively. It is then apparent that the number of ways of having a jog in the whole chain is three and that there is no way in which a jog can be formed simultaneously in each of the two segments of the C(18:1Δ9) chain at $T > T_m$. The total combined number of ways that a coupled rotamer ($g'tg'$ kink or $g'tttg'$ jog) can be formed in the chain as a whole and in each of the two segments of the chain is thus 20 for the sn-2 (C(18:1Δ9)) chain.

Using the same approach just described, the total number of possible ways that a coupled rotamer (kink or jog) can be formed in the sn-2 acyl chain and in the two segments simultaneously can be identified to vary from 12 to 20, 24, and 17 as the Δ-bond in the C(18:1Δ9) chain migrates from 7 to 9, 11, and 13, respectively. These numbers, of course, reflect the relative order of conformational variability of the sn-2 acyl chain at $T > T_m$. Consequently, at $T > T_m$, the conformational variability of a sn-2 acyl chain containing a Δ-bond at different positions along the chain has the following decreasing order: C(18:1Δ11) > C(18:1Δ13) > C(18:1Δ15) > C(18:1Δ17).

In the foregoing discussion, the conformational variability is expressed by the sn-2 acyl chain as an isolated chain. The presence of an adjacent saturated sn-1 acyl chain may impose nonuniform steric constraints on the sn-2 acyl chain and hence may limit the tendency of the sn-2 acyl chain to express its conformational variability in a nonuniform way at $T > T_m$. It is well known from 2H NMR studies of mixed-chain lipid bilayers at $T > T_m$ that the order parameter ($S_{CD}$) of the sn-1 saturated chain is roughly constant over the initial portion of the chain with a progressive decrease near the last four C-C bonds. This $S_{CD}$ profile indicates that the motional averaging is not uniform along the chain but is larger near the methyl end (20). This characteristic feature can be taken to imply that the last four C-C bonds in the sn-1 acyl chain are highly flexible and, hence, they do not impose much steric constraints on the neighboring acyl chain. When the chain length of the sn-1 acyl chain is considerably longer than that of the sn-2 acyl chain such as C(22):C(18:1Δ13)PE and C(20):C(18:1Δ13)PE, the first four C-C bonds of the sn-1 acyl chain do not pack immediately adjacent to the sn-2 acyl chain. In this case, the sn-2 acyl chain, including the last four C-C segments, is packed next to the more rigid portion of the sn-1 acyl chain, in which the CD bond has a relatively constant value of $S_{CD}$. Consequently, the conformational variability of the sn-2 acyl chain as a whole is limited uniformly by the neighboring sn-1 acyl chain. For these monoenoic lipids, the relative order of conformational variability of the C(18:1Δ9) chain can be expected to be virtually identical to the one derived earlier for an isolated chain. However, as the chain length of the sn-1 acyl chain becomes nearly equal to that of the sn-2 acyl chain, the last four C-C segment of the sn-1 acyl chain will then be packed next to the last four C-C segment of the sn-2 acyl chain. In this case, the conformational variability of the sn-2 acyl chain is limited nonuniformly by the neighboring sn-1 acyl chain. Specifically, the last four consecutive trans C-C bonds of the sn-2 acyl chain can be thought to have differential tendencies to form coupled isomers, being considerably less restricted toward the chain methyl terminus. Consequentially, the conformational variabilities of the four sn-2 acyl chains under comparison will have, at $T > T_m$, the following order: C(18:1Δ13) > C(18:1Δ13) > C(18:1Δ17) ≥ C(18:1Δ13) chain. Owing to the nearly identical chain lengths for the four monoenoic lipids shown in Fig. 5 (A-D), the degree of conformational variability of the lipid's sn-2 acyl chain in this series of monoenoic lipids is thus expected to exhibit a relative order as follows: C(16):C(18:1Δ3)PE > C(16):C(18:1Δ3)PE > C(16):C(18:1Δ3)PE. This order, to a first approximation, can be taken to reflect the relative transition entropy change of bilayers for these four monoenoic lipids. Based on the Clausius equality $T_m = \Delta H/\Delta S$ and the reasonable assumption of the virtually constant value of $\Delta H$ (Table I), one can expect that the order of $T_m$ values for aqueous dispersions of the four monoenoic lipids is, starting from the lowest value: C(16):C(18:1Δ11)PE < C(16):C(18:1Δ13)PE < C(16):C(18:1Δ15)PE < C(16):C(18:1Δ17)PE. Indeed, this expectation is borne out by experimental data (Figs. 1 and 2). In contrast, for the C(22):C(18:1Δ13)PE series, the chain length of the sn-1 acyl chain is considerably longer than that of the sn-2 acyl chain; hence, the conformational variability of sn-2 acyl chain will be limited nearly uniformly by the adjacent sn-1 acyl chain. The $T_m$ values of the monoenoic lipids in this series is thus expected to have the following order: C(22):C(18:1Δ11)PE < C(22):C(18:1Δ13)PE < C(22):C(18:1Δ15)PE < C(22):C(18:1Δ17)PE. Again, this expectation is borne out by experimental data (Fig. 2).

Theoretically, there are many combined ways in which single and coupled trans → gauche isomerizations can occur in the lipid's acyl chain as the lipid bilayer undergoes the gel → liquid-crystalline phase transition. In this report, we have only presented those limited examples ($g'tg'$ kink or $g'tttg'$-jog in the sn-2 acyl chain as a whole and in each of the two segments), which serve to illustrate the relative conformational variability exhibited by a series of monoenoic phosphatidylethanolamines. These examples should be considered to reflect only the relative trend and not the absolute overall conformational variability. In fact, the $\Delta S$ values associated with this series of lipids are, within experimental errors, indistinguishable (Table I), suggesting that the overall conformational variability for each member of the series induced by the phase transition is nearly the same. However, the $\Delta S$ or $\Delta H$ values must be different in order to account for the V-shaped curve shown in Fig. 2, although they may not be sensitive enough to be detected calorimetrically. Based on the results of our MM calculations and the nearly constant values of $\Delta H$ (or $-\Delta E^*$$^*$, Fig. 7) for the homologous series of lipids with identical molecular weight and identical structure of the sn-1 acyl chain, we suggest that each of the V-shaped curves observed in Fig. 2 is a result of an entropy-driven phenomenon. Moreover, the effect of the position of the Δ-bond on the $T_m$ of monounsaturated phosphatidylethanolamines can be attributed qualitatively to two factors. 1) The primary one is the number of ways in which the consecutive trans C-C bonds in the two chain segments separated by the Δ-bond can undergo coupled isomerizations, at $T > T_m$, to form kinks or jogs. 2) The secondary factor or the fine-tuning is the effective chain length difference between the two acyl chains of the mixed monoenoic lipid.

Recently, we have reported two $T_m$-structure relationships for saturated mixed-chain phosphatidylethanolamines, C(X):(Y)PE (16). One equation can be used to predict the $T_m$ values for lipid bilayers composed of C(X):(Y)PE with a longer effective sn-1 acyl chain, and the other is derived for lipid bilayers composed of C(X):(Y)PE with a longer effective sn-2 acyl chain. Through the use of these two equations, it is possible to carry out the reverse calculation and to obtain the (X,Y) values of an equivalent C(X):(Y)PE based on the $T_m$ value of a monoenoic lipid, C(X,Y;1A)n PE. For instance, the equivalent saturated C(X):(Y)PE for C(16):C(18:1Δ3)PE is C(16):C(11.3)PE. In other words, monoenoic C(16):C(18:1Δ3)PE and saturated C(16):C(11.3)PE share a common main phase tran-
sition temperature. Judging from the chain melting temperature, the effect of the introduction of a Δ-bond into the sn-2 acyl chain in a saturated phosphatidylethanolamine appears equivalently to shorten the sn-2 acyl chain. This raises an interesting question of whether the shorter equivalent saturated sn-2 acyl chain containing Y carbons can be correlated with the two nearly parallel chain segments separated by the Δ-bond in the sn-2 acyl chain of the monoenoic lipid species.

Six monoenoic phosphatidylethanolamines with a common sn-1 palmitoyl chain are shown in Fig. 5. The corresponding saturated C(16):C(Y):1Δ-PE for these C(16):C(Y):1Δ-PE lipids are indicated between brackets in Fig. 5. Clearly, there is no discernible correlation between the sn-2 acyl chain length of the equivalent saturated C(16):C(Y):1Δ-PE and the (C-C)
_{upper}

value or (C-C)
_{lower}

value of the sn-2 acyl chain of the monoenoic lipid, C(16):C(Y):1Δ-PE. However, the monoenoic lipids shown in Fig. 5 can be divided into two groups, I and II, according to their (C-C)
_{upper}

and (C-C)
_{lower}

values: Group I with a longer upper segment, and Group II with a longer lower segment. It can then be seen from Fig. 5 that, within each group, the sn-2 acyl chain length of the equivalent saturated C(16):C(Y):1Δ-PE correlates approximately with the (C-C)
_{trans}

value of the longer segment of the unsaturated sn-2 acyl chain. If the longer segments are indistinguishable with the same (C-C)
_{trans}

value, then the shorter segment with a larger (C-C)
_{trans}

value correlates with the sn-2 acyl chain of the equivalent saturated C(16):C(Y):1Δ-PE with a bigger Y' value.

The observation just described is used to derive a general equation between the \( T_a \) and the structural parameters of monoenoic phosphatidylethanolamines, C(X):C(Y):1Δ-PE, within each group. For saturated mixed-chain phosphatidylethanolamines, C(X):C(Y):1Δ-PE, the \( T_a \) value has previously been shown to relate to two structural parameters \( \Delta C \) and \( N \) for monoenoic lipids, however, two additional structural parameters, \( N_1 \) and \( N_2 \), are introduced. These two new parameters are related to the \( X \), (C-C)
_{upper}

, and (C-C)
_{lower}

values as follows: \( N_1 = (X - 1) + (C-C)_{upper} \) and \( N_2 = (X - 1) + (C-C)_{lower} \) for Group I lipids, and \( N_1 = (X - 1) + (C-C)_{upper} \) and \( N_2 = (X - 1) + (C-C)_{lower} \) for Group II lipids. These additional parameters account for the observed effective decrease in \( T_a \) as caused by the presence of the Δ-bond. Specifically, \( N_a \) refers to the sum of the trans C-C bond lengths in the sn-1 acyl chain and the trans C-C bond lengths in the longer segment of the sn-2 acyl chain, and \( N_a \) refers to the sum of the trans C-C bond lengths in the shorter segment of the sn-2 acyl chain. Furthermore, the presence of a Δ-bond in the sn-2 acyl chain of an energy-minimized structure results in a kink as shown in Fig. 5; hence, the sn-2 acyl chain length is reduced by one C-C bond length in comparison with that of the saturated chain. For monoenoic C(X):C(Y):1Δ-PE, \( \Delta C = X - Y + 2.5 \) and \( N = X + Y - 1.5 \). These relations can be derived readily based on the similar equations obtained with C(X):C(Y):PE (16) and the reduction of the sn-2 acyl chain length by one C-C bond length as caused by the Δ-bond. For instance, \( \Delta C = 1.5 \) and \( N = 38.5 \) C-C bond lengths can be calculated for C(18):C(22):1Δ-PE, where the negative sign indicates that the effective chain length of the sn-1 acyl chain is shorter than that of the sn-2 acyl chain.

Of the 30 \( T_a \) values determined in the present study, 16 are obtained with Group I monoenoic lipids with a longer upper segment in the sn-2 acyl chain, and the other 14 species belong to Group II lipids with a longer lower segment in the sn-2 acyl chain. Two general relationships can be derived for the two groups of monoenoic lipids by subjecting the \( T_a \) values of these lipids and their structural parameters to the multiple regression analysis. These two relationships are given as Equations 1 and 2 as follows.

For Group I monoenoic lipids, C(X):C(Y):1Δ-PE, with a longer upper segment in the sn-2 acyl chain,

\[
T_a = 133.72 + 4.55AC - 583.79(\Delta C/N) + 363.21AC(N + \Delta C) - 2044.54(1/N) - 197.35(1/N_2)...
\]

with a correlation coefficient (\( \delta \)) of 0.9984 and a root mean square error of 0.5614.

For Group II monoenoic lipids, C(X):C(Y):1Δ-PE, with a longer lower segment in the sn-2 acyl chain,

\[
T_a = 147.46 + 2.15AC - 612.89(\Delta C/N) + 474.17AC(N + \Delta C) - 2114.33(1/N) - 460.62(1/N_2)...
\]

with \( \delta = 0.9970 \) and a root mean square error of 0.4331.

The experimental \( T_a \) values obtained calorimetrically and the calculated \( T_a \) values obtained from Equations 1 and 2 for all monoenoic lipids under study are presented in Table I. The largest difference of 1.1°C between the experimental and the calculated \( T_a \) values is seen for C(22):C(22):1Δ-PE, corresponding to a relative error of 2.1% in °C or a relative error of 0.4% in Kelvins.

Since the calculated and observed \( T_a \) values are in excellent agreement, we are therefore confident to apply Equations 1 and 2 to obtain the \( T_a \) lines for the four homologous series of monoenoic lipids shown in Fig. 2. In this \( T_a \) versus \( \Delta \) plot, each solid connectivity line is generated from seven \( T_a \) values calculated for C(X):C(18:1Δ)-PE in which the Δ-bond moves stepwise from C(7) to C(13) position. Clearly, these calculated \( T_a \) lines are V-shaped. Moreover, the minimal \( T_a \) values for all four series of monoenoic lipids are seen to occur commonly when Δ-bonds are located between C(10) and C(11) atoms in the sn-2 acyl chains. Finally, it should be pointed out that Equations 1 and 2 ought to be employed only to estimate the \( T_a \) values for sn-1 saturated/sn-2 unsaturated monoenoic phosphatidylethanolamines with \( \Delta C \) in the range of −3.5 to +8.5 and \( \Delta C/N \) in the range of −0.87 to +0.210, since these two equations have been derived based on the \( T_a \) values of monoenoic phosphatidylethanolamines with the same appropriate ranges of \( \Delta C \) and \( \Delta C/N \).

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