Effects of Various Numbers and Positions of cis Double Bonds in the sn-2 Acyl Chain of Phosphatidylethanolamine on the Chain-melting Temperature*

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In an attempt to investigate systematically the effects of various single and multiple cis carbon-carbon double bonds in the sn-2 acyl chains of natural phospholipids on membrane properties, we have de novo synthesized unsaturated C_{20} fatty acids comprised of single or multiple methylene-interrupted cis double bonds. Subsequently, 15 molecular species of phosphatidylethanolamine (PE) with sn-1 C_{20}-saturated and sn-2 C_{20}-unsaturated acyl chains were semi-synthesized by acylation of C_{20}-lysophosphatidylcholine with unsaturated C_{20} fatty acids followed by phospholipase D-catalyzed base-exchange reaction in the presence of excess ethanolamine. The gel-to-liquid crystalline phase transitions of these 15 mixed-chain PE, in excess H_2O, were investigated by high resolution differential scanning calorimetry. In addition, the energy-minimized structures of these sn-1 C_{20s}-saturated/sn-2 C_{20u}-unsaturated PE were simulated by molecular mechanics calculations. It is shown that the successive introduction of cis double bonds into the sn-2 acyl chain of C(20):C(20:2)PC can affect the gel-to-liquid crystalline phase transition temperature, T_m, of the lipid bilayer in some characteristic ways; moreover, the effect depends critically on the position of cis double bonds in the sn-2 acyl chain. Specifically, we have constructed a novel T_m diagram for the 15 species of unsaturated PE, from which the effects of the number and the position of cis double bonds on T_m can be examined simultaneously in a simple, direct, and unifying manner. Interestingly, the characteristic T_m profiles exhibited by different series of mixed-chain PE with increasing degree of unsaturation can be interpreted in terms of structural changes associated with acyl chain unsaturation.

Most naturally occurring diacyl phospholipids in eukaryotic cell membranes are of a mixed acyl chain variety, meaning that the fatty acids esterified at the sn-1 and sn-2 positions of the glycerol backbone are originated primarily in vivo from saturated and unsaturated fatty acyl-CoA, respectively. Since the chemical composition of fatty acids can vary greatly in terms of the acyl chain length, the degree of unsaturation, and the position of cis carbon-carbon double bonds (Δ-bonds),1 membrane phospholipids are structurally an extremely diverse group of amphipathic molecules. In a given type of cell, membrane phospholipids may amount to several hundreds of distinctive chemical species. Despite the bewildering diversity, the basic motif of most unsaturated fatty acyl chains is surprisingly simple, viz. in the sn-2 acyl chain, the cis carbon-carbon double bonds are invariably separated by a three-carbon unit comprised of a methylene group (–CH_2–) sandwiched by two olefinic carbons. The biochemical significance of this regular methylene-interrupted interval is, however, not clear.

Although it has long been known that mixed-chain diacyl phospholipids in aqueous media can uniquely assemble into a two-dimensional sheet-like structure called the lipid bilayer, progress is nonetheless slow in understanding how variations in the chemical composition of fatty acyl chains affect the structure/property relationship of the lipid bilayer. This is, in part, due to the fact that up to the present time not a single x-ray crystal structure of mixed-chain phospholipid is available. A second reason is that many diacyl mixed-chain phospholipids containing single or multiple cis double bonds are rather difficult to synthesize (or semi-synthesize) in a typical biochemical or biophysical laboratory. Nevertheless, the pioneer work of Keough and co-workers (1-3) did provide interesting results showing how variations in the number of cis double bonds in the sn-2 acyl chain of phosphatidylcholine (PC) at certain fixed positions can affect the phase transition temperature (T_m) of the lipid bilayer. Specifically, Keough and associates (1-3) have shown by DSC that the introduction of a cis double bond into the sn-2 acyl chain of C(20):C(20:2)PC at carbon 11 from the carbonyl end, or C(11), gives rise to C(20):C(20:1)PC, which has a considerably lower T_m, relative to its saturated counterpart. The introduction of a second cis double bond into the sn-2 acyl chain at the methylene-interrupted position toward the methyl end yields C(20):C(20:2)C(13,14)PC with a further reduction in T_m. Interestingly, the introduction of a third cis double bond at C(17) results in a small increase in

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1 The abbreviations used are: Δ-bonds, cis-carbon-carbon double bonds; PC, phosphatidylcholine; PE, phosphatidylethanolamine; C(20):C(20:1)PC, saturated PE with 20 carbons in the sn-1 and 20 carbons in the sn-2 acyl chains; C(20):C(20:2)C(13,14)PC, monounsaturated PE with a saturated sn-1 C_{20s}-acyl chain and a monounsaturated sn-2 C_{20u}-acyl chain with a cis carbon-carbon double bond at the nth carbon atom from the carbonyl end (Δ_n); C(20):C(20:2)C_{20s,x,y}PE, PE with a saturated sn-1 C_{20s}-acyl chain and a dienoic sn-2 C_{20u}-acyl chain in which two methylene-interrupted Δ-bonds are at the n and (n + 3) carbon atoms from the carbonyl end; C(20):C(20:3)C_{20s,x,y}PE, and C(20):C(20:4)C_{20s,x,y}PE, 3, 4, and 5, respectively, methylene-interrupted Δ-bonds in the sn-2 C_{20s}-acyl chains; DSC, differential scanning calorimetry; MM, molecular mechanics; T_m, phase transition temperature; ΔT_m, phase transition peak width at half-maximal height; ATS, all-trans segment.
**T_m Diagram for PE with Δ-Bonds**

T_m for C(20):C(20:3)-dPhyPC. This down and up trend in T_m has been confirmed calorimetrically by other groups (4, 5). One can immediately raise a relevant question as to whether this down and up T_m profile is a special or a general characteristic for acyl chain unsaturation. Phrased differently, what kind of T_m profile will be observed if the first Δ-bond is introduced at C(5) or C(17) in the sn-2 acyl chain followed by successive incorporations of Δ-bonds at regular methylene-interrupted intervals, proceeding toward the methyl or carbonyl end? One may further ask an even more important question: do we know how to interpret the observed T_m profile in terms of molecular structures of unsaturated phospholipids? In order to find answers to these fundamental questions, phospholipids with Δ-bond(s) at different positions along the sn-2 acyl chain need to be synthesized first, and the synthesized lipids should then be subjected to DSC studies. Furthermore, the structures of unsaturated lipids in the bilayer at T ≪ T_m have to be estimated.

Recently, we have semi-synthesized a limited number of diacyl mixed-chain phosphatidylethanolamines (PE) with sn-1 C(20)-saturated and sn-2 C(20)-unsaturated acyl chains (6–9). Our calorimetric data showed that the T_m profile exhibited by a series of mixed-chain PE containing 1–3 cis Δ-bonds in the sn-2 C(20) acyl chains at Δ11, Δ11,14, and Δ11,14,17-positions, respectively, is parallel to that displayed by the corresponding mixed-chain PC observed earlier by Keough et al. (8). The influence of single and multiple Δ-bonds on the chain-melting behavior of PC and PE bilayers thus appears very similar. If we can delineate the common structural features governing the shapes of T_m profiles for various series of mixed-chain PE, the information obtained may provide an understanding of the structure/property relationships underlying most other naturally occurring phospholipids. With this in mind, in the present study we have extended the list of synthesized mixed-chain PEs containing sn-1 C(20)-saturated and sn-2 C(20)-unsaturated acyl chains to a total of 15 different species. The phase transition behavior of these mixed-chain PE, in excess H_2O, has also been investigated by high resolution DSC. Based on the calorimetric data, a novel T_m diagram is generated for the first time. In this diagram, T_m values of 9 series of mixed-chain PE each containing three or more lipids are systematically arranged. The shapes of T_m profiles displayed by various lipids in all nine series can be seen simultaneously in plots derived from the T_m diagram. Furthermore, in this study we have used the computer-based molecular mechanics (MM) calculations to simulate the energy-minimum structures of these mixed-chain PEs. The characteristic T_m profile obtained with lipids in each series of mixed-chain PE can be interpreted in terms of structural changes of the sn-2 acyl chain of the lipid resulting from acyl chain unsaturation.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—With the exception of six species of unsaturated C(20) fatty acids that were synthesized in this laboratory as described in the next paragraph, all other C(20)-unsaturated fatty acids including arachidonic acid were obtained from Sigma. Lysophosphatidylcholine with a C(20)-acyl chain was purchased from Avanti Polar Lipids (Alabaster, AL). Phospholipase D, type I from cabbage, was obtained from Sigma. Chemicals used for the fatty acid synthesis were supplied by Aldrich. All routine reagents and organic solvents were of reagent and spectroscopic grades, respectively, and they were obtained from various commercial sources.

**Synthesis of Unsaturated C(20) Fatty Acids and Semi-synthesis of Mixed-chain PE**—In the present investigation, six species of C(20)-unsaturated fatty acids were synthesized; they were cis-14-eicosenoic, cis-12-eicosenoic, cis,cis-5,8-eicosenadienoic, cis,cis-6,11-eicosenadienoic, cis,cis-14,17-eicosenadienoic, and all-cis-8,11,14,17-eicosatetraenoic acids. For monoenoic acids, the synthesis was carried out based on the method of Holman and co-workers (10, 11). By using the synthesis of cis-14-eicosenoic acid as an example, this method can be briefly described as follows: the starting material is 1-bromoundecan-11-ol. After the hydroxy group has been protected by 3,4-dihydro-2H-pyranyl, the primary alkyl derivative can interact with heptene-1 in the presence of butyl lithium to yield α-hydroxyl alkylene. Upon further reacting with CH_2(CO_2Et)_2 in the presence of EtONa, the chain elongation step gives a product of appropriate total number of carbons, viz. the eicosa-14,17-dieicosenoic acid. Finally, the triglyceride of the C(20)-acids was hydrogenated using Lindlar catalyst to form cis-14-eicosenoic acid. The syntheses of various dienoic C(20) fatty acids were accomplished by the established procedure published recently from this laboratory (9). For the synthesis of all-cis-8,11,14,17-eicosatetraenoic acid, the method of Osbund et al. (12) was employed, in which 1-bromoundeca-2,5,8-triynec acid was reacted with 10 mmol phosphorus pentoxide at 200 °C for 3 h. From the reaction mixture, the desired 1,4-bis(2,2-biphenyl)-1,4-diyl diacrylate was purified by column chromatography on silica gel 60, with which a mixture of CHCl_3, CH_3OH, 5% NH_4OH, 175:35:4 (v/v/v) was used as the eluant. Only a single spot was observed for each of the PE synthesized, after about 1 μmol per sample was loaded on the thin layer plate and developed in CHCl_3, CH_3OH, 5% NH_4OH (65:30:5). Prior to use, the lipid powder obtained from lyophilization of the lipid/benzene solution was kept at −20 °C.

**High Resolution DSC Measurements**—The lipid samples used for DSC experiments were prepared according to our previously reported protocols (9). Specifically, the lyophilized lipid powder was dispersed in cold aqueous buffer solution containing 50 mm NaCl, 0.25 mm diethylentriaminopentaacetic acid, 5 mm phosphate buffer, pH 7.4, and 0.02 mm NaN_3. All DSC experiments were performed on a MicroCal MC-2 calorimeter with a DA-2 digital interface and data acquisition utility for automatic collection (Microcal, Northampton, MA). In these DSC runs, a constant heating scan rate of 15 °C/h was used; lipid samples were scanned a minimum of three times with at least 60–90 min of equilibration at low temperatures between scans. As in our previous studies (6–9), the phase transition temperature and the transition enthalpy were determined from the second DSC heating curve. Specifically, the gel-to-liquid crystalline phase transition temperature, T_m, corresponds to the peak position with maximal peak height, and the transition enthalpy, ΔH, can be determined from the area under the transition peak and the lipid concentration using the software provided by Microcal. In general, the T_m values obtained at the transition with maximal peak height from the second DSC heating run was reproduced at ± 0.5 °C. The lipid sample with a ΔH value, however, showed considerable higher error owing to the uncertainty in deciding the onset and completion temperatures of the transition curve. The relative errors may amount to 20% for very broad transition curves as exhibited by some polysaturated lipids.

**Molecular Mechanics (MM) Calculations**—All molecular mechanics (MM) force field calculations were carried out using an IBM RS/6000 computer workstation. The software MM3 (version 92) for MM calculations was supplied by Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN. The MM3 computation began with the input of the estimated atomic coordinates for mixed-chain PE followed by systematic adjustment of the structural parameters by repeated automatic cycles of the Newton-Raphson minimization technique (16). These cycles of self-adjusted computation came to a halt as the steric energy reached the minimum. The structural data resulting from the MM3 computation were stored. Subsequently, these data were transferred into a Pentium P5–200 platform equipped with HyperChem 4.0 software (HyperCube, Gainesville, FL), from which the three-dimensional graphic images of the energy-minimized lipid molecules can be visualized. Details of the procedure for MM calculations for the energy-minimized structures for sn-2 unsaturated phospholipid were described previously (17–19). It should be mentioned, however, that prior to stochastic search for the energy-minimized conformation of sn-1 saturated/sn-2 unsaturated PE, the atomic coordinates (e.g. torsion angles) of the initially crude structure for a given mixed-chain PE were estimated based on the single crystal structure of C(12):C(12)PE (20) and the energy-minimized unsaturated
The Phase Transition Behavior of Lipid Bilayers Composed of PE with \( \Delta \)-Bonds

RESULTS

The Phase Transition Behavior of Lipid Bilayers Composed of PE with sn-1 Saturated C\(_{20}\) and sn-2 Unsaturated \( \omega 3 \left( \omega 6 \right) \) or \( \omega 9 \left( \omega 12 \right) \) Acyl Chains—Fig. 1 shows the second DSC heating curves for aqueous dispersions prepared individually from a saturated identical chain C(20):C(20)PE and its five unsaturated \( \omega 3 \left( \omega 6 \right) \) derivatives. These unsaturated \( \omega 3 \)PEs contain 1–5 \( \text{cis} \) \( \Delta \)-bonds in the sn-2 acyl chain of the lipid, with the position of the commonly shared double bond being 3 carbons from the methyl end (the \( \omega 3 \)- or \( \Delta 1 \)-position). The abbreviated name for each unsaturated lipid species is indicated under each transition curve, and above the transition curve, the value of \( T_{m} \) obtained with each of the six lipid dispersions is also indicated. Furthermore, the \( T_{m} \) value obtained with each individual \( \omega 3 \)PE is plotted in the inset against the number of \( \Delta \)-bonds in that lipid species.

The thermodynamic parameters associated with the chain-melting phase transitions of C(20):C(20)PE and its unsaturated \( \omega 3 \) and \( \omega 6 \) derivatives are summarized in Table I. The \( T_{m} \) for the \( \omega 3 \)PE series of C(20):C(20)PE and some of its derivatives increases as the number of double bonds is increased. For instance, the one displayed by the aqueous dispersion of C(20):C(20)PE at 17-position. The abbreviated name for each PE with sn-1 saturated C\(_{20}\) and sn-2 unsaturated \( \omega 3 \left( \omega 6 \right) \) or \( \omega 9 \left( \omega 12 \right) \) acyl chains is plotted in the inset. The \( T_{m} \) of Fig. 1, the \( T_{m} \) value is observed to decrease nonlinearly with a stepwise increase in the number of \( \text{cis} \) carbon-carbon double bonds in the sn-2 acyl chain. In particular, the \( T_{m} \) increment is diminished substantially between C(20):C(20):3\( \Delta 9,11,14 \)PE and C(20):C(20):5\( \Delta 9,11,14 \)PE. This nonlinear decrease in \( T_{m} \) reflects that the successive increase in the degree of acyl chain unsaturation has a nonadditive \( T_{m} \) lowering effect. Nevertheless, all experimental \( T_{m} \) values fit reasonably well by a least squares binomial curve with a correlation coefficient of 0.9836 (the inset of Fig. 1). Values of \( T_{m} \), \( \Delta H \), and other thermodynamic parameters associated with the gel-to-liquid crystalline (or chain-melting) phase transition for all five species of unsaturated \( \omega 3 \) derivatives of C(20):C(20)PE are summarized in Table I.

In Fig. 2, the second DSC heating curves for aqueous dispersions prepared from four unsaturated \( \omega 6 \)PEs and their common parent compound, C(20):C(20)PE, are illustrated. It is evident that all these DSC curves display single endothermic transitions each with a distinct \( T_{m} \) value. Moreover, a nonlinearly decreased \( T_{m} \) curve in the plot of \( T_{m} \) versus the number of \( \text{cis} \) carbon-carbon double bonds is observed (the inset of Fig. 2). In particular, the lipid species with an sn-2 arachidonyl (or all-cis-5,8,11,14-eicosatetraenoic) chain has the smallest \( T_{m} \) value of 6.6 °C. It should be mentioned that the phase transition behavior of C(20):C(20)PE and some of its \( \omega 6 \)-unsaturated derivatives has been reported recently from this laboratory (8). However, the recently reported DSC thermograms were incomplete; for instance, the one displayed by the aqueous dispersion of C(20):C(20):1\( \Delta 13 \)PE was missing. In contrast, Fig. 2 comprises all DSC thermograms for PE with sn-1 saturated/ sn-2 C\(_{20}\) or \( \omega 6 \)-unsaturated acyl chains. In Table 1, the values of \( T_{m} \), \( \Delta H \), and other thermodynamic parameters associated with the phase transition of C(20):C(20)PE and its four \( \omega 6 \)-unsaturated derivatives are summarized.

As described under “Experimental Procedures,” the monounsaturated C(20):C(20):1\( \Delta 4 \)PE was semi-synthesized by acylation of C(20)-lyso-PC with cis-14-eicosenoic acid followed by

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**Table I**

| Lipid             | \( T_{m} \) | \( \Delta H \) | \( \Delta S \) | \( \Delta T_{1/2} \) |
|-------------------|-------------|----------------|--------------|---------------------|
| C(20):C(20)PE     | 82.5        | 12.5           | 35.2         | 0.6                 |
| C(20):C(20):1\( \Delta 17 \)PE | 66.8        | 8.8            | 25.9         | 1.0                 |
| C(20):C(20):3\( \Delta 9,11,14 \)PE | 45.7        | 5.2            | 16.3         | 0.9                 |
| C(20):C(20):3\( \Delta 6,9,11,14 \)PE | 23.3        | 6.0            | 20.2         | 2.2                 |
| C(20):C(20):4\( \Delta 6,9,11,14 \)PE | 16.4        | 5.7            | 19.6         | 2.4                 |
| C(20):C(20):5\( \Delta 6,9,11,14 \)PE | 3.5         | 5.7            | 20.6         | 3.9                 |
| C(20):C(20):1\( \Delta 13 \)PE | 47.7        | 8.2            | 25.6         | 0.9                 |
| C(20):C(20):2\( \Delta 14 \)PE | 22.4        | 4.5            | 15.2         | 2.4                 |
| C(20):C(20):3\( \Delta 14 \)PE | 15.6        | 5.5            | 19.1         | 1.6                 |
| C(20):C(20):4\( \Delta 5,6,9,11,14 \)PE | 6.6         | 5.2            | 18.6         | 3.1                 |
Fig. 3 has the shape of a right-angled triangle, comprising 15 species of sn-1 C_{20\text{-}}-saturated/sn-2 C_{20\text{-}}-unsaturated PE. These lipids are arranged into 5 levels depending on the position of the ω-carbon, where the ω-carbon is defined as the first olefinic carbon atom in the lipid’s sn-2 acyl chain when counting from the methyl end of the chain. The five parallel levels of unsaturated lipids are layered from top to bottom according to the following order: ω15PE, ω12PE, ω9PE, ω6PE, and ω3PE. Furthermore, the unique $T_m$ value of any given sn-1 C_{20\text{-}}-saturated/ sn-2 C_{20\text{-}}-unsaturated PE is shown under the abbreviated name of the given lipid species in Fig. 3. Vertically, each column in the $T_m$ diagram also represents a series of unsaturated PEs, which share a common Δ-n bond. Hence, each series is designated as the ΔPE series, where the superscript $n$ denotes the position of the common cis carbon-carbon double bond (Δ-bond) in the sn-2 acyl chain when counting from the carbonyl end. In this case, the carbonyl carbon is designated as the first carbon, or C(1), of the acyl chain. Next, we shall see that with this $T_m$ diagram, the effects of acyl chain mono- and polyunsaturation on the chain-melting behavior of lipid bilayers can be examined directly in a unifying manner.

For lipid species aligned horizontally along each row in the $T_m$ diagram (Fig. 3), the added cis carbon-carbon double bond is introduced at a regular methylene-interrupted interval, proceeding toward the carbonyl end of the sn-2 acyl chain. In addition, this $T_m$ diagram shows another common feature exhibited by each series of the ω(3–12)PE as follows. The $T_m$ decreases continuously but nonlinearly with a stepwise increase in the number of cis double bonds. Consequently, we can arrive at a general conclusion that the gel-to-liquid crystalline phase transition behavior of PE bilayers is influenced markedly in a systematic way by the number of cis carbon-carbon double bonds present in the sn-2 acyl chain of the PE.

The lipid species vertically aligned along each column in the $T_m$ diagram also show a growing number of cis double bonds in the sn-2 acyl chain of the lipid (Fig. 3). However, the methylene-interrupted cis double bond is added on the methyl side of the existing double bond. Interestingly, the $T_m$ values exhibited by the ΔPE series of C(20):C(20:1Ω)PE, C(20):C(20:2Ω)PE, C(20):C(20:3Ω)PE, and C(20):C(20:4Ω)PE show a linear relationship with the number of cis double bonds. Consequently, we can arrive at a general conclusion that the gel-to-liquid crystalline phase transition behavior of PE bilayers is influenced markedly in a systematic way by the number of cis carbon-carbon double bonds present in the sn-2 acyl chain of the PE.
The most important conclusion that can be drawn from the diagram illustrated in Fig. 3 is that the number and the position of cis double bonds in the sn-2 acyl chain of PE can characteristically influence the gel-to-liquid crystalline phase transition temperature of the PE bilayer. To recapitulate this important point further, all $T_m$ values of C(20):C(20)PE derivatives shown in Fig. 3 are plotted three-dimensionally against the number and the position of cis double bonds. Specifically, in the plot of Fig. 4A, $\omega(n)$-carbon represents the first olefinic carbon at the position of $n$ from the methyl end, where the superscript $n$ denotes the position of the first olefinic carbon in the sn-2 acyl chain when counting from the carbonyl end. All together, there are 15 molecular species of unsaturated mixed-chain PE in this $T_m$ diagram, and their $T_m$ values are given under the abbreviated names of the corresponding mixed-chain PE.

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on the atomic coordinates of the single crystal structures of saturated diacyl phospholipids and unsaturated fatty acids, the energy-minimized structures of some unsaturated phospholipids in the crystalline state bilayer have been simulated by molecular mechanics (MM) calculations (18, 19). This MM approach is employed in the present study to construct the minimum energy structures for various PEs with sn-1 \( \text{C}_{20} \)-saturated \( \text{sn}-2 \) unsaturated acyl chain and a short chain segment separated by the \( \Delta^2 \)-bond in the \( \text{sn}-2 \) acyl chain of \( \text{C}(20)\text{:(C}(20)\text{)}\text{L}^\text{2})\text{PE} \).

In Fig. 5, the energy-minimized structures of six lipid species in the \( \omega \text{3PE} \) series are presented using space-filling and wire models. These structures can be taken to approximate the monomeric lipids packed in the crystalline bilayer. Here, the head groups of all six lipid molecules are aligned identically. Furthermore, the zigzag plane of the all-trans \( \text{sn}-1 \) acyl chain of each individual lipid species is seen in the wire model to lie perpendicularly to the paper plane, whereas the \( \text{sn}-2 \) acyl chain is seen in the space-filling model to project in front of the \( \text{sn}-1 \) acyl chain. For \( \text{C}(20)\text{:(C}(20)\text{)}\text{PE} \), the segment of the \( \text{sn}-2 \) acyl chain running approximately in parallel with the all-trans \( \text{sn}-1 \) acyl chain extends from \( \text{C}(3) \) to \( \text{C}(20) \) with 17 C–C bond lengths. However, the all-trans segment (ATS) of the \( \text{sn}-2 \) acyl chain is assumed to extend from \( \text{C}(3) \) to \( \text{C}(19) \) with 17 methylene units (Fig. 5). This assumption is based on the notion that the chain terminal CH\(_2\)–CH\(_2\) bond is usually disordered at \( T < T_m \), particularly for lipids packed in the gel state bilayer. In the case of monounsaturated \( \text{C}(20)\text{:(C}(20)\text{)}\text{L}^\text{2})\text{PE} \), the sequence of the \( \Delta^1 \)-containing kink in the \( \text{sn}-2 \) acyl chain is \( s' \Delta^2 \), where \( s' \) and \( \Delta \) are shew (–) and cis double bonds, respectively. By MM calculations, a set of optimal torsion angles for this \( s' \Delta^2 \) sequence is determined to be \((-109^\circ, -1.1^\circ, -120^\circ)\) as indicated in Fig. 5. Consequently, the \( \text{sn}-2 \) acyl chain of \( \text{C}(20)\text{:(C}(20)\text{)}\text{L}^\text{2})\text{PE} \) has a crankshaft-like topology in which a long and a short chain segments separated by the \( \Delta^2 \)-bond can be identified. The long chain segment extends from \( \text{C}(3) \) to \( \text{C}(17) \) with 14 C–C bond lengths, and the short chain segment including the methyl end is only 2 C–C bond lengths long. In this configuration, we define the all-trans segment in the long chain segment of the kinked \( \text{sn}-2 \) acyl chain as ATS. In the case of \( \text{C}(20)\text{:(C}(20)\text{)}\text{L}^\text{2})\text{PE} \), the ATS has 14 consecutive methylene units as indicated in Fig. 5. It should be noted that ATS is one C–C bond length shorter than that of the long chain segment due to the fact that the C–C single bond preceding the \( \Delta^2 \)-bond has a shew (–) conformation with an optimal torsion angle of \(-109^\circ\). For polyunsaturated \( \omega \text{3PEs} \), the energetically most favorable structures obtained with MM calculations are also included in Fig. 5. Here, the \( \text{sn}-2 \) acyl chains are seen to adopt roughly an overall kinked motif. In particular, the optimal torsion angles for the sequences containing \( s' \) and \( \Delta \) bonds (the kink sequences) are given under the two molecular models of each energy-minimum structure. It should be noted from Fig. 5 that the short chain segment succeeding the \( \Delta^2 \)-bond is identical in length for all unsaturated \( \omega \text{3PEs} \); in contrast, the length of ATS preceding the kink sequence in the \( \text{sn}-2 \) acyl chain decreases progressively with increasing numbers of \( \Delta \)-bonds.

The energy-minimized structures of the five lipids listed in the right column in the \( T_m \) diagram (Fig. 3) have also been determined by MM calculations using the MM3 program (data not shown). Like lipids in the \( \omega \text{3PE} \) series shown in Fig. 5, lipids in this \( \Delta^2 \text{PE} \) series contain up to 5 methylene-interrupted cis carbon-carbon double bonds in the \( \text{sn}-2 \) acyl chain. The chemical and molecular structures of these lipids, however, differ from those in the \( \omega \text{3PE} \) series. Specifically, the cis carbon-carbon double bond of the monounsaturated lipid lies in between \( \text{C}(5) \) and \( \text{C}(6) \) near the carbonyl end, and it is designated as the \( \Delta^3 \)-bond. The polyunsaturated lipids have their methylene-interrupted double bonds added on the methyl side of the \( \Delta^2 \)-bond. As a result, the short chain segment of the kinked \( \text{sn}-2 \) acyl chain is invariable in length, 2 C–C bond lengths, extending from \( \text{C}(3) \) to \( \text{C}(5) \) for all lipids in this series. In contrast, the length of ATS is shortened progressively as the new cis double bond is added successively into the \( \text{sn}-2 \) acyl chain. This decreasing trend in ATS, in essence, identical to that observed in Fig. 5 for the \( \omega \text{3} \) series of \( \text{C}(20)\text{:(C}(20)\text{)}\text{PE} \),

The minimum-energy structures of \( \text{C}(20)\text{:(C}(20)\text{)}\text{L}^{1\text{1}}\text{PE} \),
C(20):C(20:2Δ11,14)PE, and (20):C(20:3Δ11,14,17)PE in the Δ11PE series are illustrated in Fig. 6 by space-filling and wire models. For C(20):C(20:1Δ)PE, the Δ11-double bond is seen to be in the middle of the sn-2 acyl chain, and the additional Δ-bonds are on the methyl side of the Δ11-bond. Unlike the variable length of ATS shown in Fig. 5, all three unsaturated lipid species in this series of Δ11PE share a constant chain length of ATS extending from C(3) to C(10) in the sn-2 acyl chain.

In Fig. 7, the energy-minimized structures of lipids in the ΔPE series are illustrated using space-filling and wire models. These four; not five lipid species share a common segment of 5 consecutive methylene units, extending from C(3) to C(7), in the sn-2 acyl chain. For C(20):C(20:2Δ)PE, there is a segment of 7 consecutive methylene units in the sn-2 acyl chain located between the olefinic carbon of C(12) and the methyl terminus; this long segment extends from C(13) to C(19) as defined, the ATS. In the case of C(20):C(20:3Δ)PE, a segment of 4 consecutive methylene units lies between C(15) and C(20) in the sn-2 acyl chain. The length of this segment is 1 methylene unit shorter than that of the linear segment near the interface extending from C(3) to C(7). Consequently, the longer linear segment near the interface is designated as the ATS for C(20):C(20:3Δ)PE. This figure thus serves as an example to demonstrate that for certain series of lipids the ATS may switch its location along the sn-2 acyl chain.

DISCUSSION

Prior to the discussion of the chain-melting phase transitions exhibited by aqueous lipid dispersions prepared individually from C(20):C(20)PE and its unsaturated ω3 derivatives, it is appropriate to first comment on the simulated structures of these ω3PEs as shown in Fig. 5. To a first approximation, these structures correspond to the optimal and static structures of PE molecules packed in the crystalline-state bilayer at T < T_m. Unlike molecular dynamics simulations, the MM-simulated structure does not explicitly provide information about the dynamic nature of lipid molecules. For instance, the sn-2 acyl chains of all unsaturated ω3PEs share a common chain segment of C(16)–C(17)=C(18)–C(19)–C(20), in which the C–C single bonds are all rotationally highly dynamic at T < T_m. Hence, this disordered methyl-terminal segment does not undergo the thermally induced trans → gauche isomerization at T_m. The dynamic nature of this short terminal segment is, however, not revealed by the MM-simulated structure. On the other hand, as the number of Δ-bonds in this series of ω3PE increases stepwise from 0 to 5, the length of ATS in this series of PE is shortened systematically by a methylene-interrupted interval. These static structural features, which will be used to correlate with the T_m in the rest of the “Discussion,” are clearly indicated in the MM-simulated structures as depicted in Fig. 5.

When a cis carbon-carbon double bond (Δ) is introduced into a long hydrocarbon chain, the six atoms in the immediate vicinity of the Δ-bond, C–CH=CH–C, are coplanar. Although the rotational flexibility of the Δ-bond in the six-atom unit is highly restricted at physiological temperatures, paradoxically the C–C single bond preceding or succeeding the Δ-bond is rotationally highly flexible, in terms of torsion-angle fluctuations, even at very low temperature of –10 °C (17). Hence, when a Δ-bond is introduced into the sn-2 acyl chain of C(20):C(20)PE near either the carbonyl end at the Δ3-position or the methyl end at the ω3-position, the short chain segment of the kinked sn-2 acyl chain in the gel-state bilayer at T < T_m can be reasonably assumed to be highly disordered, and hence it contains virtually no trans rotamers. We believe that this assumption is justified by its utility in the following discussion.

Fundamentally, the thermally induced gel-to-liquid crystalline phase transition of the lipid bilayer occurring at T_m involves principally the trans → gauche rotational isomerization of methylene groups about C–C single bonds along the acyl chains of the lipid (22). Since the short segment of the kinked sn-2 acyl chain is assumed highly disordered in the gel-state bilayer at T < T_m, it thus makes no contributions to the chain disordering process at T_m. However, consecutive methylene groups in both the ATS of the sn-2 acyl chain and the all-trans sn-1 acyl chain can be induced thermally to undergo the disordering process of trans → gauche isomerizations. When we compare the thermodynamic parameters (T_m, ΔH, and ΔS)
associated with the chain-melting phase transition for unsaturated lipids in the ω3PE series at \( T < T_m \), we mention primarily the length of ATS. This is due to the fact that in the gel-state bilayer an identical length of the all-trans sn-1 C\(_{20}\)-acyl chain exists in all lipids in the ω3PE series. Remember that the C–C double bond is rotationally highly restricted. We, therefore, also take the rigidity of multiple Δ-bonds into consideration. Specifically, we suggest that the C–C double bond exerts its effect on the chain-melting phase transition when the Δ-bond in the sn-2 C\(_{20}\)-acyl chain reaches the number of three.

The changes in the chain length of ATS and the number of Δ-bonds in the sn-2 acyl chain of the lipid can explain qualitatively the \( \Delta H \) trend observed with lipids in the ω3PE series as shown in Table I. Here, the \( \Delta H \) values are seen to decrease initially with increasing number of Δ-bonds. In particular, the \( \Delta H \) value is at a minimum for C(20):C(20:2\( \Delta^{4,15} \))PE with a sn-2 dienoyl chain; thereafter, \( \Delta H \) values are virtually independent of the number of Δ-bonds. The transition enthalpy associated with the chain-melting transition of the bilayer is \( \Delta H = H_{lc} - H_{gel} \), where \( H \) is the enthalpy of the lipid bilayer and the subscripts lc and gel denote the liquid-crystalline and gel phases of the lipid bilayer, respectively. It is well known that saturated PE and its sn-1 saturated/sn-2 unsaturated derivatives are highly dynamic and disordered in lipid bilayers in the liquid-crystalline state; hence, the lateral chain-chain contact interactions are minimal among lipids in these liquid-crystalline bilayers. We can thus assume that \( H_{lc} \) is virtually identical for lipids with 0–5 Δ-bonds. As a result, the \( \Delta H \) trend exhibited by lipids in the ω3PE series can, to a first approximation, be related to the \( H_{gel} \) values for these lipids. For unsaturated lipids packed in the ordered gel-state bilayer, the lateral chain-chain van der Waals attractive interactions can be directly related to the length of ATS; moreover, these interactions are also modulated by the dynamic state of the ATS. As the first two cis C–C double bonds are introduced into the sn-2 acyl chain of C(20):C(20)PE at the \( \Delta^{17} \) and \( \Delta^{14,17} \)-positions, the length of ATS decreases progressively (Fig. 5). In addition, the ATS as a whole also becomes more dynamic due to the high flexibility of the C–C single bonds adjacent to methylene-interrupted cis double bonds. Consequently, the lateral van der Waals attractive chain-chain interactions are weakened by the initial acyl chain unsaturation. The marked reduction in the \( \Delta H \) can thus be interpreted as follows: increasing up to two Δ-bonds there is a steady increase in \( H_{gel} \) as a result of progressive weakening of the overall chain-chain contact interactions. Beyond two Δ-bonds, the rigid multiple methylene-interrupted Δ-bonds are assumed to act as a structural unit in the sn-2 acyl chain which can facilitate the favorable lateral chain-chain contact interaction, thus resulting in a decrease in \( H_{gel} \). This enhanced contact interaction evidently is enough to compensate the opposing effect of ATS resulting from incorporation of additional Δ-bonds. Consequently, for lipids with 3–5 Δ-bonds in the ω3PE series, their \( H_{gel} \) and hence \( \Delta H \) values are nearly identical.

Similarly, the transition entropy for the chain-melting phase transition can be expressed as \( \Delta S = S_{lc} - S_{gel} \). For lipids in the ω3PE series, the value of \( S_{lc} \) can be assumed to decrease linearly with increasing number of Δ-bonds. This assumption is based on the fact that rotation of the C–C double bond is energetically prohibited; hence, a stepwise increase in the number of Δ-bonds corresponds to a progressive decrease in the randomness or entropy of the acyl chain of the lipid. In the gel-state bilayer, however, the effect of Δ-bonds on the \( S_{gel} \) value cannot be linear. Specifically, as the Δ-bond increases from 0 to 2, the rotational freedom of the lipid molecule as a whole or the \( S_{gel} \) value increases markedly due to the high flexibility of C–C single bonds adjacent to the Δ-bonds. Above 2, the \( S_{gel} \) decreases with increasing Δ-bonds as a result of the increased rigidity of multiple Δ-bonds. In particular, the maximal \( S_{gel} \) occurs at Δ-bonds of 2, where the C–C single bonds adjacent to Δ-bonds are highly flexible, whereas the overall
rigidity of the two Δ-bonds is not sufficient to cause a substantial decrease in $S_{pol}$. Based on the proposed linear $S_{pol}$ curve and the nonlinear $S_{pol}$ curve in the plot of $S$ versus the number of Δ-bonds, a nonlinear $\Delta S$ curve with a minimum of transition entropy at Δ-bonds of 2 can be expected for lipids in the ω6PE series. This expected $\Delta S$ trend is indeed qualitatively similar to the calculated $\Delta S$ values obtained with lipids in the ω3PE series as shown in Table I.

For lipids in the ω3PE series, the changes in $T_m$ as a function of alterations in the number of Δ-bonds can now be considered. First, the following identity holds: $T_m = M\Delta H/\Delta S$. Second, despite the scattering of the data the $M\Delta H$ and $\Delta S$ both change in the same direction as the number of Δ-bonds varies. Specifically, the $M\Delta H$ and $\Delta S$ both decrease markedly with increasing number of Δ-bonds up to 2; thereafter, they increase slightly and then remain nearly unchanged (Table I). Based on these relationships and the $T_m$ profile observed in Fig. 1, we can conclude that the origin of $T_m$ is largely enthalpic, not entropic. Hence, for each lipid in the ω3PE series the main contribution to $T_m$ is the overall lateral chain-chain attractive van der Walls interaction in the gel-state bilayer. However, in view of all lipids in the ω3PE series, particularly those with 3–5 Δ-bonds, the relative $T_m$ values must also be modulated differentially by entropic variations. As a result, the shape of the $T_m$ profile varies somewhat from that of the $M\Delta H$ profile. As discussed earlier, the chain-chain van der Walls attractive interaction depends largely on the length of ATS and the number of Δ-bonds in the sn-2 acyl chain. In addition, the small contribution of the entropic effect also relates to the ATS and Δ-bonds. Unfortunately, quantitation of the relative contributions of ATS and Δ-bonds to $T_m$ remains is not possible. Nevertheless, the relative magnitudes of $T_m$ for lipids in the ω3PE series can be correlated qualitatively with the variations in the structural parameters of ATS and Δ-bonds. We, therefore, propose that the nonlinear $T_m$ profile seen in Fig. 1 (the inset) can be reasonably attributed to the net result of the following two opposing effects as follows: 1) the $T_m$ lowering effect caused by the progressive shortening of ATS, and 2) the $T_m$ elevating effect exerted by the increasing rigidity of 3–5 Δ-bonds. It is important to point out that the shortening of a three-carbon interval in the ATS has a more pronounced $T_m$ lowering effect than the opposing effect of the added Δ-bonds for lipids in this series of ω3PE. Consequently, the $T_m$ profile seen in the inset of Fig. 1 is characterized by a decreasing, not an increasing, temperature mode. It is perhaps worth mentioning that the two opposing effects are caused paradoxically by the same structural change, viz. the increasing degree of acyl chain unsaturation in the sn-2 acyl chain.

We further postulate that the combined effects of the length of ATS and the multiple Δ-bonds on $T_m$ discussed above are also operative in bilayer membranes composed of other series of PE with 3 or more Δ-bonds. The ω6 and Δ6 series of C(20):C(20)PE derivatives illustrated in the $T_m$ diagram (Fig. 3) can serve as examples. In each of the two PE series, the monounsaturated lipid has its Δ-bond located near the methyl or carboxyl end, causing the sn-2 acyl chain kinked into two segments with unequal lengths. In particular, the long segment of the kinked acyl chain contains the highly ordered ATS. Furthermore, the incorporation of the additional Δ-bond always takes place in this long segment at a regular methylene-interrupted interval, resulting in a stepwise shortening of the ATS and hence a continuous decrease in $T_m$. However, the magnitude of the $T_m$ reduction must be damped down somewhat due to an increasing number of Δ-bonds beyond three. Specifically, the multiple Δ-bonds in the sn-2 acyl chains of lipids in these two series of PE tend to promote higher $T_m$, but this $T_m$ elevating effect is less than the $T_m$ lowering effect exerted by the shortening of the ATS in the sn-2 acyl chain. On balance, the $T_m$ values in the plot of $T_m$ versus the number of Δ-bonds are expected to fall on nonlinearly decreasing curves for lipids from the ω6-, or Δ6-PE series. This expectation is indeed borne out by experimental data (Fig. 2, inset, and Fig. 3).

The monounsaturated C(20):C(20:1Δ11,13)PE is a common lipid species shared by the ω9PE and the Δ13PE series as shown in the $T_m$ diagram (Fig. 3). The topological feature of C(20):C(20:1Δ13)PE is illustrated in Fig. 6f. In particular, the kinked cis-11-eicosenoyl chain consists of two roughly parallel segments, the upper and lower segments, joined by a kink sequence of $s'$–$g$'. Here, the upper segment designates the chain segment closer to the bilayer/H2O interface, and the lower segment is assigned to the one containing the methyl group. In the kinked sn-2 cis-11-eicosenoyl chain of C(20):C(20:1Δ13)PE, the ATS with 8 methylene units is located in the upper segment, extending from C(3) to C(10). The lower segment, however, has 7 methylene units extending from C(13) to C(19). If a new Δ-bond is incorporated successively into the cis-11-eicosenoyl chain at the regular methylene-interrupted interval, this process will yield two different series of unsaturated PE. Specifically, the ω6PE and the Δ13PE series are obtained if the incorporation of Δ-bonds takes place in the upper and lower segments of cis-11-eicosenoyl chain, respectively. Interestingly, the $T_m$ profiles exhibited by these two series of unsaturated PE are distinctly different (Fig. 4, A and B), indicating that the position of the incorporated Δ-bond can markedly affect the chain-melting behavior. The shape of each of the two $T_m$ profiles will be interpreted later in terms of the length and location of ATS and the rigidity of the multiple Δ-bonds.

In the gel-state bilayer, the polymethylene units in the upper chain segment of the acyl chain of the lipid are more ordered than those in the lower segment near the bilayer center. This is due largely to the fact that the upper segment is linked covalently at both ends, and the lower segment has a free and dynamic methyl terminus. The lateral chain-chain contact interactions are thus stronger for upper chain segments. Consequently, ATS in the upper chain segment can contribute somewhat more to the overall chain disordering process of trans → gauche isomerizations in comparison with the equivalent length of ATS located in the lower chain segment. Hence, when two lipids with the same length of ATS are compared, the one with ATS in the upper segment will have a higher $T_m$. Similarly, if a short segment of consecutive methylene units is in the upper portion of the sn-2 acyl chain and its length differs from that of ATS in the lower segment by only one or two –CH2– units, then this short segment can most likely undergo the thermally induced trans → gauche isomerizations. It thus contributes to the overall chain-melting process. With these basic concepts in mind, we can rationalize the characteristic $T_m$ profile exhibited by lipids in the ω9PE series.

The three lipids in the ω9PE series, C(20):C(20:1Δ11)PE, C(20):C(20:2Δ8,11)PE, and C(20):C(20:3Δ8,11)PE, share a common lower segment of 7 consecutive methylene units in their sn-2 acyl chains. As shown in Figs. 6f and 7H, the C(20):C(20:1Δ11)PE → C(20):C(20:2Δ8,11)PE conversion is accompanied by two distinctive structural changes as follows: the length of ATS is shortened by one –CH2– unit, and the position of ATS is shifted from the upper to the lower chain segment. Both changes can result in a lowering of $T_m$. Indeed a decrease of 13 °C in $T_m$ is observed experimentally to accompany such a conversion (Fig. 3). The third member of the ω9PE series is the trienoic C(20):C(20:3Δ8,11)PE. Although the MM-simulated structure is not shown, this trienoic lipid contains an ATS that is identical to the corresponding ATS in the dioenoic C(20):C(20:2Δ8,11)PE. Despite the identical position and length of ATS, the
The energy-minimum structures of the three lipids in the $\Delta^{11}$PE series are illustrated in Fig. 6. One common feature shared by them is the identical length of the ATS located in the upper chain segment. As a result, the length of ATS alone cannot account for the variations of $T_m$ observed with lipids in the $\Delta^{11}$PE series; other structural features have to be considered. For C(20):C(20):11PE, the lower segment of the sn-2 acyl chain has 7 consecutive methylene units (Fig. 6D). One of these –CH$_2$- units, particularly those located far away from the chain methyl end, can make contributions to the chain-melting process of trans $\rightarrow$ gauche isomerizations, resulting in a higher $T_m$. The other two lipids in the same series are C(20):C(20):2$\Delta^{11}$PE and C(20):C(20):3$\Delta^{11,14,17}$PE, in which the short lower segments are relatively disordered at $T < T_m$. Moreover, their lengths are smaller than the corresponding short segment in C(20):C(20):11PE by 3 and 6 C–C methylene units, respectively, as shown in Fig. 6. Among the three lipids in the $\Delta^{11}$PE series, C(20):C(20):11PE must, therefore, exhibit the highest $T_m$. Next, we continue the comparison between C(20):C(20):2$\Delta^{11,14}$PE and C(20):C(20):3$\Delta^{11,14,17}$PE. Structurally, the fundamental difference between them lies in the number of $\Delta$-bonds. Earlier, we have postulated that the presence of three to five $\Delta$-bonds in the sn-2 C$\text{sn-2}$ acyl chain of PE can cause the bilayer to exhibit a higher $T_m$. As before, this $T_m$ elevating effect is small in comparison with the opposing effect exerted by the reducing length of the chain segment during acyl chain unsaturation. This is, however, not applicable in the case of the C(20):C(20):2$\Delta^{11,14}$PE $\rightarrow$ C(20):C(20):3$\Delta^{11,14,17}$PE conversion.

In particular, the number of consecutive methylene units present in the short lower segments of cis,cis,11,14-eicosadienoyl chain is 4 only (Fig. 6). This short lower segments in the gel-state bilayer is thus highly disordered, and it makes no contributions to the chain-melting process of trans $\rightarrow$ gauche isomerizations underlying the main phase transition at $T_m$. In going from C(20):C(20):2$\Delta^{11,14}$PE to C(20):C(20):3$\Delta^{11,14,17}$PE, a $\Delta$-bond is introduced into this highly disordered segment of the sn-2 acyl chain at C(17), causing a further shortening of the lower segment. Since this segment is highly disordered prior to the conversion, a shortening of this segment by 3 –CH$_2$- units upon unsaturation at C(17) will not affect appreciably the $T_m$.

As a result, the opposing effect of the rigid triple $\Delta$-bonds of C(20):C(20):3$\Delta^{11,14,17}$PE becomes dominant, and the relative magnitude of $T_m$ between C(20):C(20):2$\Delta^{11,14}$PE and C(20):C(20):3$\Delta^{11,14,17}$PE thus becomes apparent. For the three lipids in the $\Delta^{11}$PE series, their $T_m$ can thus be rationalized to have the following decreasing order: C(20):C(20):1$\Delta^{11}$PE $>$ C(20):C(20):3$\Delta^{11,14}$PE $>$ C(20):C(20):2$\Delta^{11,14}$PE. This order will yield a down and up $T_m$ curve in the plot of $T_m$ versus the number of $\Delta$-bonds.

The $\Delta^{9}$PE series consists of the following four lipid species: C(20):C(20):1$\Delta^{9}$PE, C(20):C(20):2$\Delta^{9,11}$PE, C(20):C(20):3$\Delta^{9,11,14}$PE, and C(20):C(20):4$\Delta^{9,11,14,17}$PE. All of them share a common upper chain segment of 5 consecutive methylene units extending from C(3) to C(7) in the sn-2 acyl chain (Fig. 7). During the C(20):C(20):1$\Delta^{9}$PE $\rightarrow$ C(20):C(20):2$\Delta^{9,11}$PE conversion, the number of –CH$_2$- units in the ATS of the sn-2 acyl chain decreases from 10 to 7, with the location of ATS being in the lower chain segment. A reduction in $T_m$ is thus expected to accompany the conversion, and such a reduction has indeed been observed calorimetrically. The subsequent C(20):C(20):2$\Delta^{9,11}$PE $\rightarrow$ C(20):C(20):3$\Delta^{9,11,14}$PE conversion is characterized by a further shortening of three –CH$_2$- units in the ATS of cis,cis,8,11-eicosadienoyl chain, leading to a continuous decrease in $T_m$. In accompanying the C(20):C(20):3$\Delta^{9,11}$PE $\rightarrow$ C(20):C(20):3$\Delta^{9,11,14}$PE conversion, the ATS shifts its location from the lower to the upper chain segment (Fig. 7, II and III).

This has an important implication, meaning that the subsequent incorporation of the fourth $\Delta$-bond into all-cis-8,11,14-eicosatrienoyl chain at C(17) affects only the length of the lower chain segment. In particular, the length and position of ATS in the sn-2 acyl chain remain unchanged as shown in Fig. 7, IV. Consequently, the trienoic C(20):C(20):3$\Delta^{8,11,14}$PE and the tetraenoic C(20):C(20):4$\Delta^{8,11,14,17}$PE share a common length of ATS in their sn-2 acyl chains’ upper segments. On the basis of the identical length of ATS alone, the $\Delta T_m$ associated with the C(20):C(20):3$\Delta^{8,11,14}$PE $\rightarrow$ C(20):C(20):4$\Delta^{8,11,14,17}$PE conversion would be zero. On the other hand, the number of $\Delta$-bonds in the sn-2 acyl chain of C(20):C(20):4$\Delta^{8,11,14,17}$PE is higher than that in C(20):C(20):3$\Delta^{8,11,14}$PE. As proposed earlier, this additional $\Delta$-bond may raise the $T_m$ of C(20):C(20):4$\Delta^{8,11,14,17}$PE. Hence, on the basis of the $T_m$ elevating effect of multiple $\Delta$-bonds and the identical length of ATS, a positive $\Delta T_m$ can be expected to underlie the C(20):C(20):3$\Delta^{8,11,14}$PE $\rightarrow$ C(20):C(20):4$\Delta^{8,11,14,17}$PE conversion. Indeed, the expected effect is borne out by DSC data presented in Fig. 3, in which the $T_m$ value displayed by the aqueous dispersion of C(20):C(20):4$\Delta^{8,11,14,17}$PE is seen to be 0.8 °C higher than that of C(20):C(20):3$\Delta^{8,11,14}$PE.

Three series of mixed-chain PEs with fixed numbers of $\Delta$-bonds can be seen along the diagonal lines in the $T_m$ diagram (Fig. 3). The monoenoic PE series has a total number of five lipids. The di- and trienoic PE series consist of four and three lipids, respectively. In response to changes in the position of the double bond, the $T_m$ values of lipids with a fixed number of double bonds give rise to a roughly V-shaped $T_m$ profile (Fig. 4, A and B). Molecular interpretations of such a characteristic $T_m$ profile have been given in detail elsewhere from this laboratory (9). Hence, we shall not discuss the V-shaped $T_m$ profiles exhibited by mono-, di-, and trienoic PE.

To sum up, for a series of sn-1 saturated/sn-2 unsaturated mixed-chain PE containing different numbers of $\Delta$-bonds, a continuously decreasing $T_m$ profile is generally observed in the plot of $T_m$ versus the number of $\Delta$-bonds as exemplified by data.
shown in Figs. 1 or 2. However, there are exceptions. For instance, the C(20):C(20:2Δ11,14)PE → C(20):C(20:3Δ11,14,17)PE and the C(20):C(20:3Δ8,11,14)PE → C(20):C(20:4Δ8,11,14,17)PE conversions are coupled with increased Tm, and the Tm profiles observed with lipids in the Δ8- and Δ11PE series are thus characterized by a down and up trend. The mixed-chain PE that can, upon unsaturation, convert into a higher Tm species has the following structural characteristics: 1) the sn-2 acyl chain contains at least two methylene-interrupted cis Δ-bonds; 2) the number of consecutive methylene units in the upper chain segment is no fewer than that in the lower chain segment; 3) the Δ-bond to be further incorporated into the unsaturated sn-2 acyl chain must be added in the lower chain segment in the direction toward the methyl terminus. Furthermore, for mixed-chain PE with 20 carbon atoms in the sn-2 acyl chain, it is interesting to note that only ω3 lipids such as C(20):C(20:3Δ11,14,17)PE and C(20):C(20:4Δ8,11,14,17)PE exhibit higher Tm values than their ω6 precursors as shown calorimetrically in the present and previous studies (8, 23). The significance of the down and up Tm profile is that it means the polyunsaturated ω3 lipid with its multiple Δ-bonds positioning near the chain terminus is highly ordered. Consequently, the central region of the bilayer’s hydrocarbon core becomes less dynamic by the presence of ω3 lipids. This is likely to promote locally a much more favorable environment for stronger lipid/protein lateral interactions. Such an environment may be critical for the stability and/or the optimal function of certain bilayer spanning proteins.

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