In an attempt to examine the effects of different numbers and positions of cis double bonds in the sn-2-acyl chain of phosphatidylethanolamine (PE) on the bilayer’s melting behavior, 21 molecular species of PE were first semisynthesized, and their Tm and ΔH values were subsequently determined by high resolution differential scanning calorimetry. In the plot of Tm versus the number of the cis double bond, some characteristic profiles were observed for the various series of PEs. For instance, if the cis double bond was first introduced into the sn-2-acyl chain of C(20):C(20)PE at the δ-position, the Tm was observed to reduce drastically. Subsequent stepwise additions of up to five cis double bonds at the methylene-interrupted positions toward the methyl end resulted in a progressive yet smaller decrease in Tm. If, on the other hand, the cis double bonds were introduced sequentially at the δ, 11,14, and 14,17-positions along the sn-2-acyl chain of C(20):C(20)PE, the Tm profile in the Tm versus the number of the cis double bond showed a down-and-up trend. Most interestingly, for positional isomers of C(20):C(20):3,5,11,14,17)PE, C(20):C(20: 3,5,11,14)PE, and C(20):C(20:3,11,14,17)PE, an inverted bell-shaped profile was detected in the plot of Tm against the position of the α-carbon for these isomers. Similar Tm profiles were also observed for C(18): C(20)PE, C(20):C(18)PE, and their unsaturated derivatives. This work thus demonstrated that both the positions and the numbers of cis double bonds in the sn-2 acyl chain could exert noticeable influence on the gel-to-liquid crystalline phase transition behavior of the lipid bilayer. Finally, a molecular model was presented, with which the behavior of the gel-to-liquid crystalline phase transition observed for lipid bilayers composed of various sn-1-saturated/sn-2-unsaturated lipids can be rationalized.

In recent years, there has been increasing evidence suggesting the existence of a close correlation between some vital functions of cells and the various degrees of unsaturation in the sn-2-acyl chains of membrane phospholipids (1–3). Consequently, detailed and systematic investigation of the properties of sn-1-saturated/sn-2-unsaturated phospholipids in the organized membrane structure such as the lipid bilayer should be recognized and pursued. Furthermore, now is the time for studying unsaturated phospholipids, since sophisticated computer-based molecular modeling techniques from the hardware and the software fields have come together to provide valuable structural and energetic information about self-assembled biomolecules within a reasonable time frame. Results obtained with experimental and modeling studies of sn-1-saturated/sn-2-unsaturated phospholipids could eventually help illuminate the specific roles played by various natural phospholipids in many membrane-related cell functions or diseases.

The first detailed and systematic studies of the thermotropic phase behavior of phospholipids containing various numbers of cis double bonds in their sn-2-acyl chains were performed by Keough and co-workers (4–6). By using two series of phosphatidylcholines (PCs) and by applying the differential scanning calorimetry (DSC) techniques, they have shown that the introduction of a cis double bond into the sn-2-acyl chain at or near the center of the chain has a marked reducing effect on the phase transition temperature (Tm) associated with the gel-to-liquid crystalline phase transition of the lipid bilayer. A second cis double bond incorporated at the methylene-interrupted position toward the methyl end also reduces the Tm, but by a smaller amount. Interestingly, the introduction of a third cis double bond between the second cis double bond and the methyl end can cause a slight increase in Tm. This calorimetric work has been confirmed more or less by other investigators using PCs with different chain lengths (7, 8).

Recently, we have studied systematically the effect of different positions of single cis double bond in the sn-2-acyl chain on the phase transition behavior of lipid bilayers composed of sn-1 saturated/sn-2-monounsaturated PCs or phosphatidylethanolamines (PEs) by high resolution DSC and computer-based molecular mechanics (MM) simulations (9–11). The position of the single cis double bond (δ) is found in both lipid classes to exert a characteristic influence on the gel-to-liquid crystalline...
phase transition temperature. Specifically, in the plot of $T_m$ versus $\Delta^\alpha$, an inverted bell-shaped profile is observed, and the minimum $T_m$ occurs when the single cis double bond is positioned at or near the center of the sn-2-acyl chain. Furthermore, based on the results of MM simulations, we have developed a molecular model that can explain the characteristic $T_m$ profile observed for monoenoic phospholipids (10, 11).

In this study, we now extend our previous work by investigating the gel-to-liquid crystalline phase transitions of one-component lipid bilayers prepared from 21 molecular species of PE; these lipids contain 0–5 cis double bonds at different positions in the sn-2-acyl chain. Specifically, we have examined the influence of the positions of the cis double bonds, in addition to the numbers of the cis double bond, on the phase transition temperature of the lipid bilayer by high resolution DSC techniques. In the plot of $T_m$ versus the number of the cis double bond, some characteristic profiles are observed for the various series of PEs with different positions of cis double bonds. Most interestingly, two parallel inverted bell-shaped $T_m$ profiles are detected in the plot of $T_m$ versus the position of the $\omega$-carbon for two sets of positional isomers of sn-1-saturated/sn-2-triunsaturated PEs. In addition, we have codified and analyzed the published $T_m$ values for lipid bilayers composed of various sn-1-saturated/sn-2-unsaturated PCs. The $T_m$ dependence of the numbers and positions of the cis double bonds in the sn-2-acyl chains of these PCs shows a similar characteristic profile that parallels the behavior of the corresponding PEs used in this study. This work thus demonstrates that both the positions and the numbers of cis double bonds in the sn-2-acyl chain can exert distinct influence on the chain melting behavior of the PE and PC bilayers. Moreover, we now expand the simple molecular model proposed earlier for monoenoic lipids with different positions of the single cis double bond in the sn-2-acyl chain. In particular, this expanded molecular model takes the rigidity of multiple cis double bonds into consideration, and it can be used to explain adequately the characteristic chain melting behavior of lipid bilayers containing various numbers and positions of cis double bonds in the sn-2-acyl chains.

**EXPERIMENTAL PROCEDURES**

**Semisynthesis of Unsaturated Phosphatidylethanolamines**—In this study, 21 molecular species of PE were semisynthesized. Three of them were saturated PE, and seven of them were either sn-1-saturated/sn-2-monounsaturated PE or sn-1-saturated/sn-2-diunsaturated PE. The semisynthesis of these 10 lipids were carried out using the established procedures as published previously (9, 12, 13). The other 11 molecular species were sn-1-saturated/sn-2-polyunsaturated PE containing three, four, and five cis double bonds at different positions in the sn-2-acyl chain. Initially, 11 molecular species of the corresponding sn-1-saturated/sn-2-polyunsaturated PC were semisynthesized and purified by established procedures (14), with a modified condition that all procedures were carried out as far as possible in an O$_2$-free, N$_2$ atmosphere to minimize autoxidation. This precautious step was also applied to the rest of the PE synthesis. The purified PCs (purity is greater than 98%) were then converted to PEs by transphosphatidylation with phospholipase D in the presence of excess amounts of ethanolamine hydrochloride, at pH 7.4, according to the procedure of Comfurius and Zwill (15) as previously published (12). The products were purified and separated by silica gel column chromatography, with which a mixture of CHCl$_3$, CH$_3$OH, 5% NH$_4$OH (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 pmol/sample was loaded on the thin layer plate. Possible oxidation of unsaturated fatty acyl chains was checked routinely as described earlier (13).

**High Resolution DSC Measurement**—DSC studies were performed on a Microcal MC-2 microcalorimeter with a DA-2 digital interface and data acquisition utility for automatic collection (Microcal, Inc., Northampton, MA), or a Hart 7708 differential scanning calorimeter (Hart Scientific, Pleasant Grove, UT). In all experiments, a constant heating scan rate of 15 °C/h was used, and 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 (9–11), the gel-to-liquid crystalline phase transition temperature and transition enthalpy were obtained from the second DSC heating curve. Specifically, the gel-to-liquid crystalline phase temperatures ($T_m$) were taken from the transition peaks at the maximum peak height positions, and transition enthalpies ($\Delta H$) were calculated from the peak areas using software provided by Microcal or Hart Inc.

**RESULTS**

**The Gel-to-Liquid Crystalline Phase Transition Behavior of Bilayers Composed of C(20):C(20)PE and Its Eight Unsaturated Derivatives**—We have examined calorimetrically the gel-to-liquid crystalline phase transition behavior of aqueous lipid dispersions prepared individually from C(20):C(20)PE and its eight unsaturated derivatives. These nine lipid species have a common structural feature; both the sn-1- and sn-2-acyl chains contain 20 carbon atoms. The structural difference among these nine lipid species lies in the numbers and/or positions of the cis double bonds in their sn-2-acyl chains. Fig. 1 illustrates the first and the immediate second DSC heating curves obtained with the aqueous lipid dispersions prepared individually from these nine lipid species. It is evident that some lipid samples exhibit thermal history-dependent phenomena. An example is demonstrated by the C(20):C(20)PE sample. Initially, two endothermic peaks centered at 66.3 and 82.7 °C are observed in
the first DSC heating scan as shown in Fig. 1. In general, the formation of phospholipid bilayers in the crystalline state requires a pronounced incubation time at temperatures considerably below \( T_m \). In our studies, phosphatidylethanolamines constituting the bilayers in the aqueous dispersion are always preincubated in the cold room (0 °C) for a minimum of 24 h. Hence, the lipid bilayers in the aqueous dispersion have already transformed into the highly ordered crystalline state prior to the first DSC heating scan. As a result, the low and high temperature peaks observed for C(20):C(20)PE in the first DSC heating scan correspond to the crystalline-to-gel and gel-to-liquid crystalline phase transitions, respectively (16). On immediate reheating, only the high temperature peak at 82.5 °C is observed calorimetrically for the same C(20):C(20)PE sample. Subsequent repeated reheating results in, again, a single endothermic transition at 82.5 °C, indicating that the gel-to-liquid crystalline phase transition of the C(20):C(20)PE bilayer is reproducible, and the crystalline-to-gel phase transition is irreversible under the present experimental conditions. Another example pertaining to the thermal history dependence of the phase transition behavior is observed for the aqueous dispersion of C(20):C(20:2\_6)PE. In the first DSC heating scan, a single, relatively sharp transition peaked at 26.4 °C is observed; however, the same sample exhibits a broader transition centered at 22.4 °C upon immediate reheating (Fig. 1). A similar thermal history-dependent phase transition behavior is also exhibited by the sample of C(20):C(20:3\_11,14,17)PE with a single, sharp transition centered at 27.2 °C in the first DSC heating scan and a broader transition peaked at 23.3 °C in the second DSC heating scan (Fig. 1). In fact, such a thermal history-dependent behavior has been observed previously for bilayers composed of saturated mixed chain PE (17) or saturated identical chain PE containing short fatty acyl chains (18, 19). Based on the DSC results of these earlier studies, the single, sharp endotherm detected in the initial DSC heating scan of the C(20):C(20:2\_11,14)PE and C(20):C(20:3\_11,14,17)PE dispersions shown in Fig. 1 can be ascribed to the crystalline-to-liquid crystalline phase transition, and the broad low-temperature transition observed in the immediate second and subsequent DSC heating scans shown in the same figure can be assigned as the gel-to-liquid crystalline phase transition. Because of the reproducible nature of the endothermic peak exhibited by the lipid sample in the second and subsequent DSC heating scans, only the values of the phase transition temperature (\( T_m \)) and the transition enthalpy (\( \Delta H \)) associated with the single endotherms observed in the second DSC scans for various lipid samples were measured; these experimentally determined values are summarized in Table I.

The effects of numbers and positions of \textit{cis} double bonds in the \textit{sn}-2-acyl chain of C(20):C(20)PE on the phase transition temperature are illustrated in Fig. 2. When the first \textit{cis} double bond is introduced near the carboxyl end of the \textit{sn}-2-acyl chain at the \( \Delta^5 \) position, the \( T_m \) value is greatly reduced from 82.5 to 58.2 °C with respect to the \( T_m \) of the saturated counterpart (Fig. 2A). When three \textit{cis} double bonds are present at the \( \Delta^6,\Delta^8,\Delta^{11} \) positions, the \( T_m \) is decreased by 35.2 °C from 58.2 to 23 °C. As methylene-interrupted \textit{cis} double bonds are further introduced sequentially into the \textit{sn}-2-acyl chain of C(20):C(20:3\_11,14,17)PE on the methyl side of the newly added \textit{cis} double bond, the \( T_m \) value is observed to decrease continuously, but by diminishing incremental changes, from 23 to 6.6 and then to 3.5 °C (Fig. 2A). A similar \( \Delta H \)-lowering trend caused by each additional unsaturation is also observed for this series of lipids (Table I). It should be noted that in this series of PE, the first \textit{cis} double bond of all unsaturated \textit{sn}-2-acyl chains begins at a common position of \( \Delta^6 \) near the carboxyl end, and all subsequent \textit{cis} double bonds are introduced into the chain segment located between the existing double bond and the methyl terminus of the \textit{sn}-2-acyl chain.

In the second series of C(20):C(20)PE derivatives presented in Fig. 2B, the first \textit{cis} double bond is inserted at the \( \Delta^{14} \) position in the \textit{sn}-2-acyl chain. In contrast to the unsaturated lipids shown in Fig. 2A, second and subsequent \textit{cis} double bonds are always introduced into the chain segment located in between the existing double bond and the carboxyl end. Hence, the \textit{sn}-2-acyl chains of this series of unsaturated phosphatidylethanolamines belong to the \( \omega-6 \) (or \( n-6 \)) family. The \( T_m \) value is shown in Fig. 2B to decrease steadily with increasing numbers of \textit{cis} double bonds; however, the incremental change of the \( T_m \) becomes progressively smaller. For comparison, the \( T_m \) values of C(20):C(20)PC, C(20):C(20:2\_11,14)PC, and C(20):C(20:3\_11,14,17)PC reported by Keough et al. (6) are also plotted against the number of \textit{cis} double bonds as indicated by the dotted curve in Fig. 2B. Most interestingly, the \( T_m \)-lowering effect of each additional unsaturation appears remarkably similar to each other for the \( \omega-6 \) PE and \( \omega-6 \) PC series of lipids. In Fig. 2B, the \( T_m \) values for C(20):C(20:1\_4)PC and C(20):C(20:1\_14)PE bilayers, 26.9 and 46.1 °C, respectively, are calculated from equations derived previously for monoenoic PC and PE (20). Other \( T_m \) values of the various \( \omega-6 \) PEs are experimentally obtained as listed in Table I. The enthalpy change (\( \Delta H \)) of the phase transition as a function of numbers of \textit{cis} double bonds follows a general trend similar to that observed for \( T_m \). The values of both \( T_m \) and \( \Delta H \) are summarized in Table I.

A third series of PEs is presented in Fig. 2C. The first \textit{cis} double bond of all unsaturated PEs in this series of lipids is positioned near the center of the \textit{sn}-2-acyl chain at the \( \Delta^{11} \) position. Second and subsequent \textit{cis} double bonds are always introduced into the \textit{sn}-2-acyl chain at a common position of \( \Delta_{13} \) near the carboxyl end. As shown in Fig. 2C, the \( T_m \) of the C(20):C(20:1\_13)PE bilayer is 39.2 °C lower than that of its saturated counterpart. A further decrease of 20.9 °C in \( T_m \) is observed in going from the C(20):C(20:1\_13)PE bilayer to the C(20):C(20:2\_11,14)PE bilayer. In contrast, a slight increase in \( T_m \) (0.9 °C) is detected as the third \textit{cis} double bond is subsequently added into the dienoic \textit{sn}-2-acyl chain on the methyl end of the \( \Delta^{14} \) double bond. The \( T_m \) values of C(20):C(20)PC and its various unsaturated derivatives have

**Table I**

| Lipid | \( T_m \) | \( \Delta H \) | \( \Delta S \) |
|-------|----------|----------|----------|
| C(20):C(20)PE | 82.5 | 12.5 | 35.2 |
| C(20):C(20:1\_4)PE | 58.2 | 8.3 | 25.1 |
| C(20):C(20:2\_11,14)PE | 43.3 | 8.1 | 25.6 |
| C(20):C(20:3\_11,14,17)PE | 22.4 | 4.5 | 15.2 |
| C(20):C(20:3\_11,14,17)PE | 23.3 | 6.0 | 20.2 |
| C(20):C(20:3\_11,14,17)PE | 15.6 | 5.5 | 19.1 |
| C(20):C(20:3\_11,14,17)PE | 23.0 | 5.8 | 19.6 |
| C(20):C(20:3\_11,14,17)PE | 6.6 | 5.2 | 18.6 |
| C(20):C(20:3\_11,14,17)PE | 3.5 | 5.7 | 20.6 |
| C(20):C(20:1\_8)PE | 75.8 | 11.2 | 32.1 |
| C(20):C(20:1\_8)PE | 33.9 | 7.0 | 22.8 |
| C(20):C(20:1\_8)PE | 7.2 | 3.1 | 11.1 |
| C(20):C(20:1\_8)PE | 10.4 | 4.7 | 16.6 |
| C(20):C(20:1\_8)PE | 3.9 | 5.5 | 19.5 |
| C(18):C(20)PE | 79.1 | 11.5 | 32.7 |
| C(18):C(20:1\_8)PE | 39.5 | 7.4 | 23.7 |
| C(18):C(20:1\_8)PE | 18.5 | 3.9 | 13.4 |
| C(18):C(20:1\_8)PE | 21.9 | 5.4 | 18.4 |
| C(18):C(20:1\_8)PE | 11.7 | 5.1 | 17.9 |
| C(18):C(20:1\_8)PE | 19.8 | 5.2 | 17.8 |
| C(18):C(20:1\_8)PE | 1.3 | 4.5 | 16.4 |
Melting Behavior of Unsaturated Phospholipids

Number of double bonds / sn-2 acyl chain

FIG. 2. The plot of the gel-to-liquid crystalline phase transition temperature ($T_m$) versus the numbers of cis double bonds in the sn-2-acyl chains of sn-1-saturated/sn-2-unsaturated PEs. The position of the first cis double bond is indicated by $\Delta^n$, where the superscript $n$ indicates the position of the first olefinic carbon atom counting from the carboxyl end. If the sn-2-acyl chain has more than one single cis double bond, the positions of the other cis double bonds are indicated in the superscript of $\Delta$ following the value of $n$. (A) All saturated PEs have a common first cis double bond at the $\Delta^5$ position. B, all unsaturated lipids belong to the $\omega$-6 family. C, all unsaturated lipids have their first cis double bond at or near the center of the sn-2-acyl chain. The dotted lines shown in B and C connect the $T_m$ values of PC as indicated.

been determined calorimetrically by Keough et al. (6). In addition, the $T_m$ values of C(18):C(18)PC and its unsaturated derivatives with the first cis double bond located near the center of the sn-2-acyl chain at the $\Delta^5$ position have been reported recently by Sanchez-Migallon et al. (7). These two sets of calorimetric data obtained with PCs are connected by dotted lines in Fig. 2C. Clearly, these two dotted curves are strikingly similar in shape to the solid curve determined in the present study using PEs. These excellent agreements indicate that within this series of unsaturated lipids the $T_m$ values of the $\omega$-3-unsaturated lipid with three cis double bonds is noticeably higher than that of the $\omega$-6-unsaturated lipid with two cis double bonds, regardless of the nature of the lipid’s head group. Since the general trends of the variations in $T_m$ as a function of the numbers of cis double bonds are different for the various series of lipids shown in Fig. 2, A, B, and C, it is thus concluded that the positions of cis double bonds, in addition to the numbers of cis double bonds, can influence the chain melting behavior of the lipid bilayer.

To further substantiate the influence of the positions of cis double bonds in the sn-2-acyl chains of PEs on the gel-to-liquid crystalline phase transition behavior of the PE bilayer, the $T_m$ and $\Delta H$ values of bilayers composed of C(20):C(20):3$\Delta^{5,8,11}$PE, C(20):C(20):3$\Delta^{8,11,14}$PE, and C(20):C(20):3$\Delta^{11,14,17}$PE are compared in Fig. 3. These three lipids species are positional isomers; their numbers of carbon atoms and numbers of cis double bonds are equal. The positions of the three cis double bonds in the sn-2-acyl chain, however, are different, and the difference can be represented by the position of the first olefinic carbon atom counting from the methyl end (or the $\omega$-carbon). As shown in Fig. 3B, the $T_m$ values of these positional isomers have an inverted bell-shaped dependence on the position of the $\omega$-carbon, with the minimal $T_m$ of 15.6 °C occurring at the $\omega$-6-position. To the best of our knowledge, this is the first time that such an inverted bell-shaped curve has been observed for the $T_m$ values of lipid bilayers composed of positional isomers of polyunsaturated phospholipids. Previously, it has been shown that the $T_m$ values of sn-1-saturated/sn-2-monounsaturated PCs and PEs with different positions of the single cis double bond exhibit inverted bell-shaped profiles in the plot of $T_m$ versus the position of the single cis double bond in the sn-2-acyl chain (9, 10). The $T_m$ profile shown in Fig. 3B thus demonstrates that, like monoenoic lipids, the melting behavior of trienoic lipids is influenced in a distinctive manner by the position of multiple cis double bonds along the sn-2-acyl chain.

The Gel-to-Liquid Crystalline Phase Transition Behavior of Bilayers Composed of C(18):C(20)PE, C(20):C(18)PE, and Their Unsaturated Derivatives—In Fig. 1, the two acyl chains of various PEs all have 20 carbon atoms. To further investigate the influence of the introduction of increasing numbers of cis double bonds into the sn-2-acyl chain with a common position of the first cis double bond, we have utilized the high resolution DSC techniques to investigate the phase transition behavior of mixed chain C(18):C(20)PE, C(20):C(18)PE, and their unsaturated derivatives. In these mixed chain PEs, the two acyl chains are of unequal numbers of carbons; however, C(18):C(20)PE...
and C(20):C(18)PE are positional isomers in which the numbers of carbons in the \(sn_1\) and \(sn_2\)-acyl chains of the first lipid species are equal to those in the \(sn_2\)- and \(sn_1\)-acyl chains, respectively, of the second lipid species.

Like the unsaturated derivatives of C(20):C(20)PE shown in Fig. 1, lipid bilayers prepared from some of the unsaturated derivatives of mixed chain PE also exhibit different phase transition behavior in the first and the second DSC heating scans (data not shown). For instance, the C(18):C(20:2 \(D_{11,14}\))PE bilayer exhibits a single, narrower endotherm peaked at 24.2 °C in the first DSC heating scan, and this we ascribe to the crystalline-to-liquid crystalline phase transition. Upon reheating, this taller endotherm is replaced by a broader transition centered at 18.5 °C, and this is assigned as the gel-to-liquid crystalline phase transition. Similarly, we find that the single transition that appears in the crystalline-to-liquid crystalline phase transition of the C(20):C(18:2 \(D_{9,12}\))PE bilayer is narrower, with a \(T_m\) of 12.4 °C in the initial DSC heating scan. Upon immediate reheating, the same bilayer exhibits a broader gel-to-liquid crystalline phase transition with \(T_m\) of 7.2 °C. The bilayer composed of trienoic \(\omega-3\) lipids such as C(18):C(20:3 \(D_{11,14,17}\))PE or C(20):C(18:3 \(D_{9,12,15}\))PE also shows a similar thermal history-dependent behavior. Nevertheless, the single transition that appears in the second DSC heating scan is always reproducible upon repeated reheating, and we assign it as the gel-to-liquid crystalline phase transition. In Table I, the \(T_m\) and \(\Delta H\) values of C(18):C(20)PE, C(20):C(18)PE, and their unsaturated derivatives are obtained from the gel-to-liquid crystalline phase transitions that appeared in the second DSC heating scans.

Fig. 4A depicts the plot of \(T_m\) versus the increasing numbers of cis double bonds in the \(sn_2\)-acyl chain of C(18):C(20)PE. In this series of unsaturated PE, the common cis double bond in the \(sn_2\)-acyl chains is located at the \(D_{14}\) position, and the additional double bonds are on the carboxyl side of the existing double bond; hence, this series of unsaturated lipids belongs to the \(\omega-6\) family. The data shown in Fig. 4A demonstrate clearly the \(T_m\)-lowering effect of each additional unsaturation. This decreasing trend in \(T_m\) is virtually identical to that of the solid \(T_m\) curve shown in Fig. 2B for the unsaturated derivatives of C(20):C(20)PE. We should point out here that the \(T_m\) value of the C(18):C(20:1 \(D_{14}\))PE bilayer, 44.5 °C, is a calculated one (20). The literature values of \(T_m\) for bilayers composed of C(18):C(20)PC, C(18):C(20:1 \(D_{14}\))PC, C(18):C(20:2 \(D_{11,14}\))PC, C(18):C(20:3 \(D_{8,11,14}\))PC, and C(18):C(20:4 \(D_{5,8,11,14}\))PC are 60.4, 25.1, -5.4, -9.3, and -13.2 °C, respectively (8, 17, 20). These \(T_m\) values are connected by a dotted line in the plot of \(T_m\) versus the number of double bonds as shown in Fig. 4A. Interestingly, this dotted curve appears remarkably similar in shape to the solid curve obtained with C(18):C(20)PE and its unsaturated derivative. It should be noted that, as described earlier, the solid and dotted curves connecting \(T_m\) values of PEs and PCs, respectively, in Fig. 2B, are also roughly parallel, suggesting strongly that the \(T_m\)-lowering effects of chain unsaturations in both PE and PC bilayers perhaps proceed by a common mechanism.

In Fig. 4B, the \(sn_2\)-acyl chains of the unsaturated derivatives of C(18):C(20)PE have a common cis double bond at the \(D_{11}\) position; in addition, the second and subsequent methylene-interrupted cis double bonds are inserted between the existing double bond and the methyl end of the \(sn_2\)-acyl chain. In the plot of the \(T_m\) versus the number of cis double bonds, the \(T_m\)
value decreases initially with increasing numbers of cis double bonds. Beyond two cis double bonds, the magnitude of $T_m$ increases slightly. Specifically, the $T_m$ of the trienoic $\delta$-3 lipid, C(18):C(20:3 $\Delta^{11,14,17}$)PE, is 21.0 °C, which is 2.5 °C higher than that of the dienoic $\delta$-6 lipid, C(18):C(20:2 $\Delta^{11,14}$)PE. As expected, the same down-and-up trend in $T_m$ is also observed in Fig. 4B for the mixed chain C(16):C(18)PC series, in which the first cis double bond in the shorter sn-2-acyl chain is inserted at the $\Delta^9$ position and the subsequent additions of cis double bonds are on the methyl side of the existing double bond. The $T_m$ values of C(16):C(18)PC and its unsaturated derivatives that appeared in Fig. 4B were those reported by Hernandez-Borrell and Keough (21) and McCabe et al. (22).

The $\Delta H$ and $T_m$ values of lipid bilayers prepared from C(18):C(20:3 $\Delta^{5,8,11}$)PE, C(18):C(20:3 $\Delta^{8,11,14}$)PE, and C(18):C(20:3 $\Delta^{11,14,17}$)PE are plotted in Fig. 3, A and B, against the position of the $\omega$-carbon of the three positional isomers. An inverted bell-shaped $T_m$ profile is observed in Fig. 3B with the minimal $T_m$ of 11.7 °C occurring at the $\omega$-position. The structural difference among these three isomers lies only in the position of the methylene-interrupted multiple cis double bonds in the sn-2-acyl chain. The observed $T_m$ profile thus provides further strong evidence indicating that the positions of cis double bonds alone can exert a noticeable influence on the lipid bilayer's transition behavior.

Fig. 5, A and B, show the variations of $T_m$ as a function of the number of cis double bonds in the sn-2-acyl chains of C(20):C(18)PE and its unsaturated derivatives. In Fig. 5A, the continuous downshift in $T_m$ arising from a series of $\omega$-3 PEs with increasing numbers of cis double bonds is, in fact, another recurring theme that has been observed repeatedly for $\omega$-6 PEs and PCs as depicted in Figs. 2B and 4A. Here, 46.9 °C is calculated as the $T_m$ value for the C(20):C(18:1 $\Delta^{15}$)PE bilayer (20). The $T_m$ curve illustrated in Fig. 5B, on the other hand, shows a somewhat more complicated, yet familiar, picture; the $T_m$ of C(20):C(18)PE decreases steadily with increasing numbers of cis double bonds, reaching a nadir at two cis double bonds. Thereafter, the incremental difference in $T_m$ changes its sign, resulting in a $T_m$ curve with a characteristic down-and-up trend. It should be noted that the same down-and-up trend in $T_m$ has already been shown to associate with the gel-to-liquid crystalline phase transition behavior of some other series of PE and PC as illustrated in Figs. 2C and 4B. Let us focus now upon the common sequence of events shared by these different series of phospholipids, leading to the characteristic down-and-up trend. First, the introduction of the initial cis double bond into the saturated sn-2-acyl chain of PE or PC occurs at or near the center of the acyl chain. Second, the subsequent incorporation of methylene-interrupted cis double bonds takes place between the existing double bond and the methyl terminus of the sn-2-acyl chain. Third, the lipid species showing an upward trend with a positive incremental change in $T_m$ is inevitably an $\omega$-3 lipid.

**DISCUSSION**

When the lipid bilayer prepared from a one-component phospholipid species undergoes the thermally induced gel-to-liquid crystalline phase transition, an equilibrium state is reached.
between the gel phase and the liquid crystalline phase at the
phase transition temperature, \( T_m \). Assuming the transition to
be a first-order equilibrium transition, the \( T_m \) is then equal to
the ratio of the transition enthalpy and the transition entropy
as follows:
\[
T_m = \frac{\Delta H}{\Delta S}
\]
Based on the experimentally determined values of \( T_m \) and \( \Delta H \), the value of \( \Delta S \) can be calculated accordingly. In Table I, the values of \( T_m \), \( \Delta H \), and \( \Delta S \) for the
one-component lipid bilayers prepared individually from sev-
eral series of PEs are summarized. It is evident that within
each series of PEs the magnitude of \( T_m \), \( \Delta H \), and \( \Delta S \) are clearly
dependent on the numbers and positions of cis double bonds in
the sn-2-acyl chains. Moreover, when the \( T_m \) value is dimin-
ished as a result of chemical modification of the sn-2-acyl chain,
the values of the accompanying \( \Delta H \) and \( \Delta S \) are, in general, also
observed to decrease. This means a greater contribution of the
energy term \( \Delta H \) to the magnitude of \( T_m \), since the other term,
\( \Delta S \), works against \( \Delta H \) in the relationship of
\[
T_m = \frac{\Delta H}{\Delta S}
\]
Consequently, as a first approximation, the term \( T_m \) may be
expressed explicitly as a function of some structural parame-
ters that are directly related to the energetic term \( \Delta H \), without
invoking the \( \Delta S \) term. Specifically, the gel-to-liquid crystalline
phase transition is accompanied structurally by a highly coop-
erative process involving the trans \( \rightarrow \) gauche isomerizations of
the C–C single bonds along the two acyl chains within each
lipid molecule in the bilayer. The calorimetrically determined
\( \Delta H \), on the other hand, represents a measure of heat required
to overcome the energy barrier for the gel-to-liquid crystalline
phase transition. This heat must therefore depend on the total
number of C–C trans bonds, which in turn is related directly to
the effective chain lengths of the lipid’s acyl chains in the
gel state bilayer. As a result, the variation in \( T_m \) for lipid bilayers
prepared individually from a series of positional isomers for a
given lipid species may be correlated with the change in the
effective acyl chain lengths of these positional isomers. Based
on this possible correlation and the general molecular structure
of monoenoic lipid obtained from MM calculations, a simple
molecular model has been developed to describe the gel-to-
liquid crystalline phase behavior of bilayers prepared from a
series of monounsaturated lipids with different positions of the
single cis double bond in the sn-2-acyl chain (9–11); specifi-
cally, three basic assumptions underlying this simple model
have been proposed, and they are briefly summarized in the
next paragraph. Subsequently, an expanded molecular model
is further proposed. The phase transition behavior of lipid
bilayers composed of multiple cis double bonds as presented
under “Results” can then be rationalized.

The three basic assumptions underlying the simple molecu-
lar model are as follows: 1) the monoenoic sn-2-acyl chain in the
sn-1-saturated/sn-2-monounsaturated phospholipid molecule
is assumed to adopt, at \( T < T_m \), an energy-minimized crank-
shaft-like motif in the gel state bilayer; hence, it consists of a
longer chain segment and a shorter chain segment separated
by the cis double bond; 2) the longer segment and the neigh-
boring all-trans sn-1-acyl chain run in a parallel manner with
favorable van der Waals attractive distance between them; and
3) the shorter segment is considered to be partially disordered
at \( T < T_m \), analogous to the molten polypeptide chain of pro-
teins, thus playing a relatively insignificant role in the attrac-
tive van der Waals chain-chain interactions in the gel state
bilayer. With this model, it can be appreciated that the total
number of C–C trans bonds in the two acyl chains of monoun-
saturated lipids in the gel state bilayer is considerably smaller

![Graphs A and B](image-url)

**Fig. 5.** The \( T_m \) versus the number of cis double bonds for bilayers prepared from C(20):C(18)PE and its derivatives. A, the unsaturated lipids belong to the \( \omega-3 \) family. B, the first cis double bond begins at the \( \Delta^\alpha \) position.
than that of the saturated counterparts due to the third assumption that the short segment is already partially disordered at $T < T_m$. As a result, the heat required to induce the cooperative process of trans $\rightarrow$ gauche isomerizations of C–C single bonds in the two acyl chains of self-assembled monounsaturated lipid molecules is decreased appreciably, resulting in a significantly lower $T_m$ value. In addition, we can use the same molecular model to explain the inverted bell-shaped $T_m$ profile observed for bilayers of monoenoic phospholipids in the plot of $T_m$ versus the position of the single cis double bond in the sn-2-acyl chain. As stated in the second assumption, the longer chain segment of the kinked sn-2-acyl chain is proposed to undergo a favorable van der Waals contact interaction with the all-trans sn-1-acyl chain in the gel state bilayer. This contact interaction energy must then depend on the effective chain length of the longer segment. When the single cis double bond is positioned at the center of a sn-2-acyl chain, the effective chain length of the longer segment has a minimum length, which is almost equal to that of the shorter segment. Hence, the van der Waals contact interaction with the all-trans sn-1-acyl chain in the gel state bilayer is also minimal. As the single cis double bond migrates away successively from the chain center toward either the carboxyl or the methyl end, the effective length of the longer segment is progressively increased, leading to a proportionally increased van der Waals interaction and hence a gradual increase in $T_m$ and $\Delta H$. The inverted bell-shaped profile of $T_m$ as a function of the location of the cis double bond can thus be envisioned based on the simple structural model. Specifically, we can take a saturated sn-2-acyl chain containing 20 carbons in C(20):C(20)PC as an example. Here, the effective chain length of this sn-2-acyl chain in the gel state bilayer of C(20):C(20)PC is about 17.5 C–C bond lengths. This effective length is approximately 1.5 C–C bond lengths shorter than that of the fully extended chain, resulting from the initial sharp bend of the sn-2-acyl chain at the C(2) position. Now, if a single cis double bond is inserted at the $\Delta^{11}$ position, the kinked monoenoic acyl chain is then shortened by about one C–C unit (11). The effective length of the longer segment preceding the cis double bond at the $\Delta^{11}$ position is 8.5 C–C bond lengths, and that of the shorter segment succeeding the cis double bond is 8 C–C bond lengths (Fig. 6). If the single cis double bond migrates from the $\Delta^{11}$ position toward the carboxyl end and stops at the $\Delta^5$ position, the effective chain lengths of the longer and shorter segments are changed to 14 and 2.5 C–C lengths, respectively (Fig. 6). On the other hand, if the single cis double bond migrates to the $\Delta^{14}$ position, the longer and shorter segments will have 11.5 and 5 C–C bond lengths, respectively (Fig. 6). Clearly, if a simple correlation between the $T_m$ and the effective chain length of the longer segment of the sn-2-acyl chain is assumed to exist for this series of positional isomers, the $T_m$ value of C(20):C(20:1$D_n$)PC isomers should have the following increasing order: $\Delta^{11} < \Delta^{14} < \Delta^5$. Indeed, this trend of $T_m$ is borne out by experimental and computational data (20). This means that our proposed simple correlation between the $T_m$ and the effective chain length of the longer segment of the monoenoic sn-2-acyl chain for this series of positional isomers is reasonable.

Now we can expand the molecular model discussed above...
and then apply the expanded model to lipids containing more than one single cis double bond. First of all, it should be mentioned that when a second cis double bond is introduced into the monoenoic sn-2-acyl chain at the methylene-interrupted position, the dienoic chain can still adopt the crankshaft-like motif as indicated by MM calculations (13). In addition, the effective length of the kinked sn-2-chain is reduced by another C-C unit, and the axes of the two chain segments separated by the two cis double bonds are roughly parallel (13). Furthermore, the vertical distance separating the two parallel axes of the chain segments is larger than that observed for monoenoic chain (13, 23), leading to a weaker lateral chain-chain interaction between the sn-1- and sn-2-acyl chain in the gel state bilayer. In comparison with other sn-1-saturated/sn-2-polyunsaturated lipids, we propose that in the gel state bilayer the overall lateral chain-chain interaction is minimal for bilayers composed of sn-1-saturated/sn-2-diunsaturated lipids. This proposal is based on a poignantly paradoxical nature of the cis double bond: the cis double bond itself is rotationally immobile, whereas the two C–C single bonds adjacent to the cis double bond are highly flexible (23). More specifically, the rigidity and the flexibility of the sn-2-acyl chain at and around the cis double bond can work against each other in terms of lateral chain-chain interactions. When there are two cis double bonds, the dienoic sn-2-acyl chain can be considered to be highly dynamical as a result of the flexible nature of the C–C single bonds adjacent to the two cis double bonds, thus resulting in a weaker lateral chain-chain interaction between the sn-1- and sn-2-acyl chains. After the second cis double bond, however, the rigidity of the multiple cis bonds is considered to play a more dominant role, thus promoting the lateral chain-chain interaction. In fact, for sn-1-saturated/sn-2-polyunsaturated lipids packed in the gel state bilayer, the multiple methylene-interrupted cis double bonds are assumed to form a structural unit with highly restricted mobility. Consequently, for a series of positional isomers of lipids containing the same number of multiple cis double bonds, the change in $T_m$ as a function of the position of the immobile unit in the sn-2-acyl chain can be expected to decrease with the corresponding change of the chain length of the longer segment of the kinked sn-2-acyl chain. The $T_m$ profile should, therefore, have an inverted bell-shaped characteristic, similar to that observed for monoenoic lipids with different positions of the single rigid cis double bond. And indeed, this characteristic profile is observed experimentally as shown in Fig. 3B.

We reemphasize that our expanded molecular model is constructed on the basis of the following two additional assumptions: 1) the sn-2-acyl chain containing two cis double bonds is highly flexible in the gel state bilayer, leading to a weakest lateral chain-chain interaction in comparison with other sn-1-saturated/sn-2-polyunsaturated lipids; 2) when the sn-2-acyl chain contains three or more cis double bonds, however, these methylene-interrupted cis double bonds can be considered as an essentially immobile unit in the gel state bilayer. Based on the expanded molecular model, the variations in $T_m$ for various unsaturated lipids with different positions of the cis double bonds can be readily correlated with the effective chain lengths of the longer segments of the sn-2-acyl chains. For instance, the effective chain lengths of the longer segments of the sn-2-acyl chains for C(20):C(20:1)$^{\Delta 14}$PE, C(20):C(20:2)$^{\Delta 11,14}$PE, C(20):C(20:3)$^{\Delta 8,11,14}$PE, and C(20):C(20:4)$^{\Delta 5,8,11,14}$PE in the gel state bilayer are 11.5, 8.5, 5.5, and 5 C–C bond lengths, respectively (Fig. 6). As expected, the $T_m$ also shows a trend that roughly parallels the chain length variation of the longer segment of the kinked chain (Fig. 2B). In Fig. 2C, a down-and-up trend of the $T_m$ profile is observed for C(20):C(20:1)$^{\Delta 11}$PE, C(20):C(20:2)$^{\Delta 11,14}$PE, and C(20):C(20:3)$^{\Delta 11,14,17}$PE. The lengths of the longer segments of the sn-2-acyl chains of this series of lipids are identical, being 8.5 C–C bond-lengths (Fig. 6). However, the rigidity of the sn-2-acyl chain containing three cis double bonds is greater than that of the dienoic sn-2-acyl chain, leading to a somewhat stronger lateral chain-chain interaction between the sn-1- and sn-2-acyl chains and hence a slightly higher $T_m$. Likewise, the general trend of the experimental $T_m$ values of C(18):C(20)PE derivatives observed in Fig. 4A also follows, in a parallel manner, the variation in the apparent chain length of the longer segment of the sn-2-acyl chain among the various lipids (Fig. 6). The down-and-up trend of $T_m$ observed in Fig. 4B, however, can be explained by the weakest chain-chain interaction proposed for dienoic lipids in the gel state bilayer.

For the various C(20):C(18)PE derivatives containing 18 carbons and various numbers and positions of cis double bonds in the sn-2-acyl chain, the effective lengths of the longer and shorter segments of the sn-2-acyl chains in the gel state bilayer are illustrated in Fig. 7. The experimental curves in the two plots of $T_m$ versus the numbers of cis double bonds shown in Fig. 5, A and B, can also be explained satisfactorily based on the variations in the chain length of the longer segment of the sn-2-acyl chain among the various lipids as depicted in Fig. 7 and the increased rigidity of the sn-2-acyl chain resulting from an increased number of cis double bonds. Finally, it should be emphasized that the simple correlation between the $T_m$ and the chain length of the longer segment of the sn-2-acyl chain in the gel state bilayer proposed in this study for various series of sn-1-saturated/sn-2-unsaturated PE (or PC) should be used with caution. It can be applied only to describe qualitatively the trend of $T_m$ variations as shown in Figs. 2–5. It should not be applied to estimated the magnitude of $T_m$ in any of the $T_m$ profiles.

CONCLUSIONS

Phosphatidylyethanolamines with a given pair of sn-1-saturated/sn-2-unsaturated acyl chains packed in the lipid bilayer can undergo a gel-to-liquid crystalline phase transition upon heating. The thermodynamic behavior of this phase transition is different from that displayed by saturated counterparts. In general, the values of $T_m$, $\Delta H$, and $\Delta S$ associated with the gel-to-liquid crystalline transition are noticeably smaller for the bilayer composed of unsaturated lipid species.

The change in $T_m$ upon the introduction of additional cis double bonds into the sn-2-acyl chain depends on both the numbers and the positions of cis double bonds along the sn-2-acyl chain.

In the presence of cis double bonds, the sn-2-acyl chain of sn-1-saturated/sn-2-unsaturated PE (or PC) is proposed to have a crankshaft-like motif at $T < T_m$. With this motif, the sn-2-acyl chain appears to consist of two chain segments with nearly parallel axes separated by the cis double bond(s). Furthermore, the longer segment and the neighboring all-trans sn-1-acyl chain are assumed to undergo favorable van der Waals interactions, whereas the shorter segment is assumed to be partially disordered at $T < T_m$.

Based on the structural model proposed above, the trend of $T_m$ variations for a series of sn-1-saturated/sn-2-unsaturated PE (or PC) in the plot of $T_m$ against the numbers of cis double bonds...
bonds can be correlated directly with the change in the effective length of the longer segment of the kinked chain. If the lengths of the longer segments are identical for a series of lipids, an increase in the rigidity of the polyunsaturated \textit{sn}-2-acyl chain should be taken into consideration. In this case, the dienoic lipid has a minimal $T_m$ value; thereafter, the $T_m$ increases slightly with increasing numbers of \textit{cis} double bonds.

The worth of a molecular model lies in its predictive power. With our molecular model, we predict that in the plot of $T_m$ versus the position of two \textit{cis} double bonds for a series of positional isomers of \textit{sn}-1-saturated/\textit{sn}-2-diunsaturated PE (or PC), the $T_m$ profile should exhibit an inverted bell-shaped characteristic. Only future experiments with dienoic lipid species can test the predictive ability of our proposed structural model.

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**FIG. 7. Schematic diagrams indicating the chain lengths of the longer and shorter segments of the kinked acyl chains, each with a total number of 18 carbons.** A C(18)-acyl chain with a maximal number of four methylene-interrupted \textit{cis} double bonds is indicated at the top. The chain lengths on both sides of the \textit{cis} double bond(s) are expressed in units of C–C bond lengths.