We have semi-synthesized 18 species of mixed chain phosphatidylethanolamines (PE) in which the sn-1 acyl chain is derived from stearic, arachidic, and behenic acids, and the sn-2 acyl chain is originated from cis,cis-octadecadienoic and cis,cis,eicosadienoic acids with the two methylene-interrupted double bonds located at various positions. These PEs constituting the bilayers in the aqueous dispersion were subjected to differential scanning calorimetric experiments. The \( T_m \) values associated with the gel-to-liquid crystalline phase transitions for these PEs are found to be significantly smaller than those of the saturated counterparts. Moreover, the magnitude of the \( T_m \)-lowering effect of acyl chain diunsaturation depends critically on the positions of the two methylene-interrupted cis double bonds in the sn-2 acyl chain. Specifically, if the sn-2 acyl chain is derived from cis,cis-octadecadienoic acid, the \( T_m \)-lowering effect has the following decreasing order: \( \Delta^{9,12} > \Delta^{6,9} > \Delta^{12,15} \). For cis,cis,eicosadienoic acyl chain, the \( T_m \)-lowering effect is stronger in the order of \( \Delta^{10,13} > \Delta^{11,14} > \Delta^{10,11} > \Delta^{8,18} > \Delta^{14,17} \). Finally, a refined molecular model is presented that can adequately explain the \( T_m \)-lowering effect of sn-2 acyl chain diunsaturation. Moreover, this same refined molecular model can also be invoked to better interpret the \( T_m \)-lowering effect observed for sn-1 saturated/sn-2 monoenoic PE.

Biological membranes are often referred to as two-dimensional sheet-like structures consisting mainly of proteins and lipids. Nevertheless, they do have a third dimension, albeit short typically in between 5 and 10 nm, in the direction normal to the membrane surface. In particular, lipids in the biological membrane are amphipathic molecules, with their long hydrophobic moieties oriented roughly parallel to the direction of the third dimension. Because of the high heterogeneity of the hydrophobic moieties, the short third dimension of the biological membrane is characterized by a nonuniform architecture. For instance, the number of the cis carbon-carbon double bond in the sn-2 acyl chain for most membrane lipids ranges from 1 to 6, with the position of the rotationally rigid cis double bond varying from the 4th carbon (\( \Delta^4 \)) when counting from the carbonyl end to the \( \omega3 \) carbon or the third carbon when counting from the methyl end of the acyl chain. These lipid molecules with various numbers and positions of cis double bonds along the sn-2 acyl chain in the direction of the third dimension of the membrane must affect the lateral lipid-lipid/lipid-protein contacts in the two-dimensional plane of the membrane which, in turn, may contribute significantly to some specific properties of biological membranes.

As an approach to understand the effects of structural variations in the third dimension of the biological membrane on the lateral lipid-lipid interactions in the two-dimensional plane of the sheet-like membrane, we have recently investigated the influence of the numbers and positions of cis C-C double bonds in the sn-2 acyl chain of phosphatidylethanolamine (PE)\(^*\) on the gel-to-liquid crystalline (or the L3 \( \rightarrow \) L1) phase transition of the lipid bilayer (1-4), a highly cooperative behavior exhibited by most, if not all, lipid molecules in the two-dimensional plane of the lipid bilayer. In particular, we have applied the high resolution differential scanning calorimetry (DSC) to follow the \( L_3 \rightarrow L_1 \) phase transition exhibited by several series of lipid species under two distinct subclasses of phosphatidylethanolamine, viz. the sn-1 saturated/sn-2 monoenoic PE and the sn-1 saturated/sn-2 trienoic PE (1-3). Our DSC results indicated clearly that the gel-to-liquid crystalline phase transitions exhibited by these two subclasses of PE share two common features (1, 3). First, the introduction of a single cis double bond or three methylene-interrupted cis double bonds into the sn-2 acyl chain lowers the phase transition temperature, \( T_m \). Second, the largest \( T_m \)-lowering effect occurs when the single or three cis double bond is located in the middle of the linear segment of the sn-2 acyl chain. Interestingly, the \( T_m \) increases progressively as the single or three cis-double bond migrates from the middle toward either end of the acyl chain (1, 3). Based on the simulated molecular structures of these unsaturated lipids packed in the gel-state bilayer as obtained by molecular mechanics (MM) calculations, we have postulated a molecular model to explain qualitatively the observed \( T_m \)-lowering effect of acyl chain unsaturation (1, 3). Explicitly, the MM-based molecular model predicts that in the plot of \( T_m \) versus the positions of two methylene-interrupted cis double bonds for a homologous series of sn-1 saturated/sn-2 dioenoic PE, the \( T_m \) profile should exhibit a V-shaped characteristic (3). In the present investigation, our main goal is to test the validity of the predicted

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1 The abbreviations used are: PE, phosphatidylethanolamine; C(\( X \))Y:PE: saturated PE with \( X \) carbons in the sn-1 and \( Y \) carbons in the sn-2 acyl chain; C(\( X \))Y(\( Y \)):PE: monounsaturated PE with a saturated sn-1 acyl chain containing \( X \) carbons and a monounsaturated sn-2 acyl chain containing \( Y \) carbons with a cis carbon-carbon double bond (\( \Delta^4 \)) at the \( n \)th carbon atom from the carbonyl end; C(\( X \))(\( Y:2\Delta^\alpha \):PE: a mixed chain PE with a saturated sn-1 acyl chain and a dioenoic sn-2 acyl chain in which two methylene-interrupted cis double bonds are at the \( n \) and \( n+3 \) carbon atoms from the carbonyl end; DSC: differential scanning calorimetry; MM, molecular mechanics; \( T_m \), phase transition temperature; \( \Delta H \), transition enthalpy; \( \Delta T_m \), transition peak width at half-maximum peak height.
V-shaped $T_m$ profile. In order to achieve this goal, a large number of sn-1 saturated/sn-2 dienoic PE needs to be synthesized. Although we have previously semi-synthesized 15 molecular species of sn-1 saturated/sn-2 dienoic PE (4), the sn-2 acyl chains of these lipids were $\omega 6$ fatty acids which can be purchased from commercial sources. Other dienoic fatty acids with the methylene-interrupted cis double bonds at $\omega 3$, $\omega 9$, and $\omega 12$ positions are not commercially available. In this study, we first synthesized these various dienoic fatty acids and then linked them via acylation to the appropriate lysolipids. Specifically, the following five series of sn-1 saturated/sn-2 dienoic PE were synthesized: C(18):(C(18:2)n,n+3)PE, C(20):(C(18:2)n,n+3)PE, C(18):(C(20:2)n,n+3)PE, C(20):(C(20:2)n,n+3)PE, and C(22):(C(20:2)n,n+3)PE. These PEs constituting the bilayers in the aqueous dispersions were then subjected to DSC experiments to study the influence of the positions of two methylene-interrupted cis double bonds on the bilayer’s $L_\alpha \rightarrow L_v$ phase transition. Another goal of this investigation is to refine the MM-based molecular model based on the $T_m$ values of sn-1 saturated/sn-2 diunsaturated PE with the two double-bond position ranging from $\Delta 5,8$ to $\omega 3$. Specifically, we attempted to obtain a simple molecular model which can adequately explain how the $T_m$ value varies as the methylene-interrupted two-double bond migrates along the sn-2 acyl chain from the $\Delta 5,8$ position near the bilayer/water interface to the $\omega 3$ position near the bilayer center.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Dienoic fatty acids with two methylene-interrupted cis double bonds at the $\omega 6$ positions such as cis,cis-12,octadecadienoic and cis,cis-11–14-eicosadienoic acids were obtained commercially from Sigma. Lyso phosphatidylcholines were supplied by Avanti Polar Lipids (Alabaster, AL). Phospholipase D, type I from cabbage, was purchased from Sigma. All routine reagents and organic solvents were of reagent and spectroscopic grades, respectively, and they were purchased from different commercial sources.

**Semi-synthesis of sn-1 Saturated/sn-2 Dienoic PE**—With the exception of $\omega 6$ dienoic fatty acids, all other dienoic fatty acids used as the starting materials for phospholipid semi-synthesis were prepared in this laboratory using modified procedures of established methods (5, 6). Basically, the synthesis of dienoic fatty acids proceeded through the coupling of relevant $\omega$-acylenic acid (HC=C(CH$_3$)$_n$-COOH) and appropriate 1-bromo-2-alkynes (R=C=CH$_2$-Br) followed by catalytic cis-hydrogenation of the triple bond using the Lindlar catalyst (6). Depending on the availability of the starting materials for making the $\omega$-acylenic acids, it is possible to synthesize the various dienoic fatty acids by two different routes. Detailed procedures will be published elsewhere; however, the outlines of the two routes are presented in Fig. 1, A and B, as reaction schemes I and II, respectively. Specifically, the synthesis of cis,cis-5,8-eicosadienoic acid, cis,cis-6,9-octadecadienoic acid, and cis,cis-8,11-eicosadienoic acid can be accomplished by the reaction scheme I, whereas that of cis,cis-12,15-octadecadienoic acid and cis,cis-14,17-eicosadienoic acid being carried out using reaction scheme II. By using high pressure liquid chromatography (7), the purity of the synthesized dienoic fatty acids can be estimated to be greater than 95%.

In this study, 18 molecular species of PE, in each of which the sn-1 acyl chain is saturated and the sn-2 acyl chain is derived from a dienoic fatty acid, were semi-synthesized and purified according to our previously published method (4). Purity was checked by UV spectroscopy, TLC, and DSC (4); the lipid powder, after lyophilization from benzene, was kept at $-20^\circ$C.

**High Resolution DSC Measurements**—The lipid samples were prepared according to established procedures (1, 4). Briefly, cold aqueous buffer solution (50 mM NaCl, 0.25 mM diethylenetriaminepentaacetic acid, 5 mM phosphate buffer, pH 7.4, and 0.02 mg/ml NaN$_3$) was added to lyophilized lipid powder to give a total lipid concentration of 2–5 mM. The exact lipid concentration was determined by phosphorus analysis. In order to avoid possible auto-oxidation of the unsaturated lipids, the cold aqueous buffer solution was degassed and then purged gently with $N_2$ gas prior to the lipid sample preparation. Furthermore, once the lipid sample was prepared, it was immediately degassed and sealed under $N_2$ followed by vortexing for about 5 min. After vortexing, the sealed lipid sample was kept at 0 °C for about 30 min and then loaded into the DSC sample cell. The lipid sample was further equilibrated in the DSC cell at a desired temperature (usually 15 °C below the $T_m$) for 120 min and then scanned. All DSC experiments were performed using a high resolution MC-2 differential scanning microcalorimeter (Microcal, Northampton, MA). Each lipid sample was scanned at least three times at a constant scan rate of 15 °C in the ascending temperature mode with at least 60–90 min of equilibration at low temperatures between scans. In order to ascertain that the same thermal history pertained to all lipid samples, only the $T_m$ value from the second DSC heating scan was reported in this study. The third DSC heating scan served as an internal control to check whether the second DSC curve was reproducibly produced. The $T_m$ value obtained at the transition peak with maximal peak height was reproduced at ±0.1 °C for each lipid sample. Details of the procedure for carrying out DSC experiments and for determining the values of the phase transition temperature ($T_m$) and enthalpy ($\Delta H$) were described in our earlier publications (1–4).

**RESULTS**

**Phase Transition Behavior of C(X):(C(18:2)$\Delta n$-n+3)PE with $X = 18$ and 20 and $n = 6, 9$, and 12**—The influence of the positions of the two methylene-interrupted cis carbon-carbon double bonds in the sn-2 acyl chain on the gel-to-liquid crystalline phase transition behavior of the lipid bilayer composed of phosphatidylethanolamines was studied by high resolution differential scanning calorimetry using two homologous series of PE, viz., C(X):(C(18:2)$\Delta n$-n+3)PE with $X = 18$ and 20 and $n = 6, 9$, and 12. Specifically, the sn-1 acyl chains of the two series PE are derived from stearic and arachidic acids, respectively, and the sn-2 acyl chains are originated from cis,cis-octadecadienoic acid with the positions of the two methylene-interrupted double bonds designated by $\Delta n$-n+3, where $n$ denotes the number of carbon atoms from the carboxyl end and equals to 6, 9, and 12. Within each series, there are three lipid species that are positional isomers. Their numbers of carbon atoms and numbers of cis double bonds are equal; however, the positions of the two methylene-interrupted cis double bonds in the sn-2 acyl chain are different. It should be mentioned at this point that the sn-2 acyl chain of saturated PE in the single crystals is bent nearly 90° at the C(2) position due to the fact that the torsional angles of C(1)-C(2) and C(2)-C(3) bonds are −119° and 65°, respectively (8). As a result, the all-trans-linear segment of the saturated sn-2 acyl chain begins at the C(3) atom and ends at the terminal methyl carbon. When two methylene-interrupted cis double bonds are introduced into the sn-2 acyl chain of PE, the relative positions of $\Delta 6,9$, $\Delta 9,12$, and $\Delta 12,15$ along the linear segment of the sn-2 acyl chain in each of the positions isomers can be schematically illustrated as shown in Fig. 2, C–E. It is apparent that the two methylene-interrupted cis double bonds, $\Delta 9,12$, of C(X):(C(18:2)$\Delta n$-n+3)PE are located nearly at the center of the linear segment of the sn-2 acyl chain, whereas the $\Delta 6,9$ double bonds of C(X):(C(18:2)$\Delta n$-n+3)PE and the $\Delta 12,15$ double bonds of C(X):(C(18:2)$\Delta n$-n+3)PE are located near the carboxyl and methyl ends, respectively, in the sn-2 acyl chains.

Fig. 3 shows the first and second DSC heating curves for aqueous dispersions prepared individually from each of the lipid species within the two homologous series of C(18):(C(18:2)$\Delta n$-n+3)PE and C(20):(C(20:2)$\Delta n$-n+3)PE with $n = 6, 9$, and 12. All six lipid samples show a single endothermic transition in the first DSC heating scans. Upon immediate second DSC heating scans, four samples display a smaller and down-shifted endothermic mode with at least 60–90 min of equilibration at low temperatures between scans. In these thermal history-dependent phenomena are identical to those typically exhibited by aqueous dispersions prepared from C(12):(C(12)PE and saturated mixed chain PE (9–12). Consequently, the high and low temperature transitions observed in the initial and subsequent DSC heating scans can be reasonably assigned as the crystal-
line-to-liquid crystalline (the $L_a \rightarrow L_a$) and the gel-to-liquid crystalline (the $L_a \rightarrow L_a$) phase transitions, respectively.

The thermodynamic parameters ($T_m$, $\Delta H$, and $\Delta T_{1/2}$) associated with the $L_a \rightarrow L_a$ phase transition for each of the six lipid samples shown in Fig. 3 are summarized in Table I. It should be mentioned that the $T_m$ value observed calorimetrically for the C(18):C(18)PE dispersion is 74.4 °C (12). This $T_m$ value is considerably higher than those of C(18):C(18:2$\Delta_1$-$\Delta_3$)PE with $n = 6, 9, 12$ as shown in Fig. 3 and Table I. Similarly, the $T_m$ value of 75.8 °C exhibited by lamellar C(20):C(18)PE is also substantially higher than the $T_m$ values observed in Fig. 3 for C(20):C(18:2$\Delta_1$-$\Delta_3$)PE with $n = 6, 9, 12$. The present study thus confirms and extends the previously published DSC results (4), indicating that the acyl chain diunsaturation mark-

**Fig. 1.** Two reaction schemes for the synthesis of dienoic fatty acids. **A**, scheme I, a synthetic route for compounds I, II, and III. **B**, scheme II, a synthetic route for compounds IV and V. 

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**Table I.** Thermodynamic parameters ($T_m$, $\Delta H$, and $\Delta T_{1/2}$) for the diunsaturated PE dispersion. 

| Compound | $T_m$ (°C) | $\Delta H$ (kcal/mol) | $\Delta T_{1/2}$ (°C) |
|----------|------------|----------------------|----------------------|
| C(18):C(18)PE | 74.4 | 4.2 | 6.2 |
| C(18):C(18:2$\Delta_1$-$\Delta_3$)PE | 71.5 | 3.8 | 5.1 |
| C(20):C(18)PE | 75.8 | 4.3 | 7.0 |

DHP -- Dihydropyran, LAED -- Lithium acetylide ethyldiamine complex, DMSO -- Dimethyl sulfoxide, THF -- Tetrahydrofuran.
membrane can result in different responses by the lipid bilayer as a whole. In this case, the different $T_m$-lowering responses have the following decreasing order: $\Delta^9\gamma > \Delta^6\gamma > \Delta^{12}\gamma$.

In Fig. 2, the structural unit of the two methylene-interrupted cis double bonds at $\Delta^9\gamma$ is seen to be nearly in the middle of the linear segment of the sn-2 acyl chain. Hence, the $T_m$-lowering effect of acyl chain diunsaturation observed in Fig. 3 appears to be similar to that of acyl chain monounsaturation (1), which has the following two characteristics. First, the $T_m$ profile has roughly the shape of the letter V. Second, the minimal $T_m$ occurs when the single cis double bond is nearly in the middle of the linear segment of the sn-2 acyl chain.

Phase Transition Behavior of C(X):C(20:2$\Delta^{n,n-3}$)PE with $X = 18, 20, 22$ and $n = 5, 8, 11, 14$—Three other series of diunsaturated phosphatidylethanolamines, in which the sn-1 acyl chains are derived from stearic, arachidic, and behenic acids, respectively, whereas the sn-2 acyl chains are originated from cis,cis-eicosadienoic acid with the two methylene-interrupted cis double bonds located at $\Delta^5\gamma$, $\Delta^8\gamma$, $\Delta^{11}\gamma$, and $\Delta^{14}\gamma$ positions, were also studied by high resolution DSC. The relative positions of these cis double bonds ($\Delta^5\gamma$, $\Delta^8\gamma$, $\Delta^{11}\gamma$, and $\Delta^{14}\gamma$) in the sn-2 acyl chains of PEs are schematically illustrated in Fig. 2, F–J. The aqueous dispersion prepared individually from some of these dienoic PEs exhibit calorimetrically the same thermal history-dependent phase transition characteristics as those shown in Fig. 3. The values of the thermodynamic parameters ($T_m$, $\Delta H$, and $\Delta T_1$) associated with the gel-to-liquid crystalline phase transition obtained from the second DSC heating scans are summarized in Table I.

Fig. 4 shows the second DSC heating curves obtained with aqueous dispersions prepared individually from 12 molecular species under the three series of C(X):C(20:2$\Delta^{n,n-3}$)PE with $X = 18, 20, 22$ and $n = 5, 8, 11, 14$. With the exception of C(22):C(20:2$\Delta^5\gamma$)PE, all thermograms shown in Fig. 4 are characterized by single and nearly symmetrical endothermic transitions peaked at distinct temperatures, indicating that our synthesized dienoic fatty acids and mixed chain PEs derived from them have high degrees of chemical and isomeric purities, respectively. To the best of our knowledge, all lipid species shown in Figs. 3 and 4, with the exception of o6 lipids, have not been studied previously by DSC or any other biophysical method. This is perhaps due to the fact that dienoic fatty acids leading to the synthesis of these PEs are not commercially available.

The $T_m$ values for aqueous dispersions of C(18):C(20)PE, C(20):C(20)PE, and C(22):C(20)PE are 79.1, 82.5, and 84.5 °C, respectively (3, 12). The $T_m$ values of the 12 lipid species, sn-1 saturated/sn-2 eicosadienoic PEs, shown in Table I are considerably smaller than the $T_m$ values of their respective saturated counterparts. A similar $T_m$-lowering effect has also been observed earlier for sn-1 saturated/sn-2 octadecadienoic PE. Consequently, the observed $T_m$-lowering effect of the sn-2 acyl chain diunsaturation can be considered as a general feature for the phase transition behavior of the lipid bilayer.

The $T_m$ for each lipid sample derived from the three series of C(X):C(Y:2$\Delta^{n,n-3}$)PE with $X = 18, 20, 22$ and $n = 5, 8, 11, 14$ can be compared in terms of the positions of two cis double bonds, $\Delta^{n,n-3}$. The $T_m$-lowering effect of acyl chain diunsaturation is stronger in the order of $\Delta^{11}\gamma > \Delta^{14}\gamma > \Delta^5\gamma > \Delta^{12}\gamma$.

The Estimated $T_m$ Values for C(X):C(20:2$\Delta^{10,13}$)PE with $X = 18, 20, 22$—The $T_m$-lowering order just described was obtained in the absence of experimentally determined $T_m$ values for C(X):C(20:2$\Delta^{10,13}$)PE. As shown in Fig. 2H, the $\Delta^{10,13}$ position in the sn-2 eicosadienoic acyl chain is nearly in the middle of the linear hydrocarbon segment of the sn-2 acyl...
In order to obtain a more precisely defined \( T_m \)-lowering order, it is necessary to include data for PE with their double bonds near the middle of the \( \text{sn}-2 \) acyl chain. Hence, we have estimated the \( T_m \) values for C(20):C(18:2\( \Delta \text{n,n} \))PE with X = 18, 20, and 22 as described below.

Recently, the gel-to-liquid crystalline phase transition temperatures for saturated, monounsaturated, and \( \text{sn}-6 \)-diunsaturated PE have been shown to correlate with some fundamental structural parameters underlying the PE molecule packed in the gel state bilayer; consequently, relatively simple equations of \( T_m \) in terms of the structural parameters can be derived (1, 4, 12). We can apply a similar approach to derive a general \( T_m \) structural parameter equation for lipid bilayers composed of dienoic PE in which the two methylene-interrupted \( \text{cis} \) carbon-carbon double bonds are in the lower half of the \( \text{sn}-2 \) acyl chain. Specifically, based on the 10 \( T_m \) values determined for C(X):C(Y:2\( \Delta \text{n,n} \))PE with X = 18, 20, and Y = 18, 20, and n = 9–14 for C(X):C(18:2\( \Delta \text{n,n} \))PE or n = 10–14 for C(X):C(20:2\( \Delta \text{n,n} \))PE.

Three structural parameters underlying the dienoic PE are defined in Fig. 5, A and B, in which molecular graphic drawings of a monomeric and a dimeric C(20):C(20:2\( \Delta \text{11,14} \))PE obtained with MM calculations are illustrated. The three defined structural parameters are abbreviated as \( \text{D} \), \( \text{SS} \), and \( \text{N} \) which specify the molecular structure of the unsaturated lipid species packed in the gel-state bilayer, and they have units of carbon-carbon bond length. The first one is \( \text{D} \), the effective chain

| Lipid | \( T_m \) | \( \Delta T_{1/2} \) | \( \Delta H \) |
|-------|-------|-------|-------|
| C(18):C(18:2\( \Delta \text{6,9} \))PE | 20.8 | 1.4 | 3.2 |
| C(18):C(18:2\( \Delta \text{9,12} \))PE | 5.7 | 1.6 | 3.0 |
| C(18):C(18:2\( \Delta \text{12,15} \))PE | 30.1 | 2.2 | 3.7 |
| C(20):C(18:2\( \Delta \text{6,9} \))PE | 22.7 | 2.7 | 3.9 |
| C(20):C(18:2\( \Delta \text{9,12} \))PE | 31.6 | 1.2 | 4.8 |
| C(20):C(18:2\( \Delta \text{12,15} \))PE | 31.6 | 1.2 | 4.8 |
| C(18):C(20:2\( \Delta \text{5,8} \))PE | 34.1 | 2.4 | 4.3 |
| C(18):C(20:2\( \Delta \text{8,11} \))PE | 27.1 | 1.1 | 4.0 |
| C(18):C(20:2\( \Delta \text{11,14} \))PE | 18.5 | 2.7 | 3.9 |
| C(18):C(20:2\( \Delta \text{14,17} \))PE | 44.9 | 1.0 | 4.8 |
| C(20):C(20:2\( \Delta \text{5,8} \))PE | 38.6 | 1.0 | 4.7 |
| C(20):C(20:2\( \Delta \text{8,11} \))PE | 30.3 | 2.1 | 4.2 |
| C(20):C(20:2\( \Delta \text{11,14} \))PE | 22.2 | 2.4 | 4.5 |
| C(20):C(20:2\( \Delta \text{14,17} \))PE | 45.7 | 0.9 | 5.2 |
| C(22):C(20:2\( \Delta \text{5,8} \))PE | 41.3 | 2.8 | 5.4 |
| C(22):C(20:2\( \Delta \text{8,11} \))PE | 34.6 | 2.8 | 5.4 |
| C(22):C(20:2\( \Delta \text{11,14} \))PE | 23.8 | 1.4 | 5.0 |
| C(22):C(20:2\( \Delta \text{14,17} \))PE | 46.8 | 1.3 | 5.6 |

FIG. 3. The first and second DSC heating scans of aqueous dispersions prepared from C(20):C(18:2\( \Delta \text{n,n} \))PE and C(18):C(18:2\( \Delta \text{n,n} \))PE, where n = 6, 9, and 12. The \( T_m \) value for each phase transition is indicated next to the corresponding thermogram. Clearly, when the two \( \text{cis} \) double bonds are located at the \( \Delta \text{n,12} \) positions, this positional isomer exhibits the smallest value of the phase transition temperature in each series of the dienoic PE.
length difference between the sn-1 and sn-2 acyl chains. Because of the sharp bend at C(2) and the presence of two cis double bonds at $\Delta^{n,n+3}$ positions, the sn-2 acyl chain is shortened by about 3.5 C-C bond lengths in comparison with its saturated counterpart (4), yielding $\Delta C = X - Y + 3.5$. The second structural parameter is SS, which defines the short segment of the kinked sn-2 acyl chain, ranging from $(n + 4)$th carbon to the terminal methyl carbon; it is related to $Y$ and $n$ as follows: SS = $Y - (n + 4)$. Finally, the thickness of the hydrocarbon core of the gel state bilayer is defined as $N$. This structural parameter represents the separation distance between the two carbonyl oxygens of the sn-1 acyl chains in the trans-bilayer dimer (Fig. 5B), and is related to $X$ and $Y$ as follows (4):

$$n = X + Y - 2.5,$$

by assuming the van der Waals contact distance between the two opposing methyl groups from the sn-1 and sn-2 acyl chains to be 3.0 C-C bond lengths. Based on simple equations of $T_m$ and structural parameters derived earlier for saturated, monounsaturated, and $\alpha$6 diunsaturated PE (1, 4, 12), one can tentatively formulate a general $T_m$ structural parameter expression for dienoic PE as shown in Equation 1.

$$T_m = a_0 - a_1 (1/N) - a_2 (\Delta C/N) + a_3 (1/SS)$$  \(\text{(Eq. 1)}\)

When experimental $T_m$ values and the computational data (N, $\Delta C$, and SS) obtained with the 10 respective lipid species shown in Table II are substituted individually into Equation 1, the resulting 10 simultaneous equations can be analyzed statistically by multiple regression method to obtain the coefficient $(a_0$, $a_1$, and $a_2)$ in Equation 1. We obtain Equation 2

$$T_m = 141.74 - 4738.93 \left(\frac{1}{N}\right) - 107.90 (\frac{\Delta C}{N}) + 81.13 \left(\frac{1}{SS}\right)$$  \(\text{(Eq. 2)}\)

with a correlation coefficient of 0.9988 and a root mean square error of 0.7009. This high value of correlation coefficient and low value of root mean square error indicate that Equation 2 is indeed an excellent $T_m$ structural parameter expression for dienoic PE. Based on Equation 2, the calculated $T_m$ values for the 10 lipid species can be obtained, and they are also presented in Table II as $T_m^{\text{cal}}$. The largest difference between $T_m^{\text{cal}}$ and $T_m^{\text{obs}}$ is 1.4 °C for C(18):C(20:2)$\Delta^{11,14}$PE, which amounts to a relatively small change of about 0.5% in kelvin. The agreement between $T_m^{\text{cal}}$ and $T_m^{\text{obs}}$ for these 10 dienoic PE is clearly excellent. Such an excellent agreement is expected, since the $T_m$ values of these 10 lipid species belong to the set from which Equation 2 was derived. Hence, comparisons between $T_m^{\text{cal}}$ and $T_m^{\text{obs}}$ have to be sought from other dienoic PEs. Table II shows the $T_m^{\text{obs}}$ values for six other lipid species ranging from C(22):C(18:2)$\Delta^{9,15}$PE to C(24):C(22:2)$\Delta^{13,16}$PE (4). The agreement between $T_m^{\text{cal}}$ and $T_m^{\text{obs}}$ values is again excellent for these six lipid species, with the largest relative difference between them being about 0.5% in kelvin. Equation 2 thus appears to be effective in predicting the $T_m$ value for mixed chain sn-1 saturated/sn-2 dienoic PE. It should be emphasized that Equation 2 was derived on the basis of experimental $T_m$ values obtained with C(22):C(18:2)$\Delta^{n,n+3}$PE with $n \geq 5$ Y; hence, it should not be used to calculate $T_m$ values for mixed chain dienoic PE with $n < 0.5$ Y.

The $T_m$ values for C(20:2)$\Delta^{11,14}$PE with $X = 18, 20,$ and 22 can be estimated from Equation 2. These values, given in Table II, can now be combined with other $T_m$ values shown in Table I to obtain more precisely defined $T_m$ profiles for the three series of C(20:2)$\Delta^{n,n+3}$PE with $X = 18, 20, 22$ and $n = 5, 8, 10, 11,$ and 14. The resulting profiles are illustrated in Fig. 6. Clearly, the dip of each of the V-shaped profiles is

![Fig. 5. Molecular graphics representations of the energy-minimized structures of monomeric C(20):C(20:2)$\Delta^{11,14}$PE (A) and transbilayer dimer of C(20):C(20:2)$\Delta^{11,14}$PE (B). The three structural parameters, SS, $\Delta C$, and $N$, described in the text are indicated. All three structural parameters have the same unit of C-C bond length. The sn-2 acyl chain is kinked. In this view, the zigzag planes of the long and short segments are seen to be nearly parallel, but not coplanar, with the lower plane extending in front of the zigzag plane of the long segment. A different view of the same kinked sn-2 acyl chain is presented in Fig. 2A.](image-url)
Melting Behavior of Diunsaturated PE

The strength of the contact interaction, however, depends implicitly on the thickness of the bilayer’s hydrocarbon segment. When the contact interaction is strong, the thickness of the bilayer’s hydrocarbon segment is small. Conversely, when the contact interaction is weak, the thickness of the bilayer’s hydrocarbon segment is large. Therefore, the thickness of the bilayer’s hydrocarbon segment affects the strength of the contact interaction.

To understand the effect of the thickness of the bilayer’s hydrocarbon segment on the strength of the contact interaction, we need to consider the all-trans and trans–gauche isomerizations of the C=C bonds. These isomerizations cause gauche–gauche transitions, which contribute significantly to the conformational disordering process.


discussion

Based on the molecular structure of monounsaturated PE obtained with molecular mechanics (MM) simulations, a molecular model has been invoked to interpret qualitatively the $T_m$-lowering effect of sn-2 acyl chain unsaturation for monounsaturated PE (1). This MM-based molecular model has been subsequently extended to explain qualitatively the similar $T_m$-lowering effect observed for trienoic PE (3). Before we present this model and apply it to interpret the $T_m$-lowering effect for dienoic PE observed in the present study, it is appropriate to mention that upon heating the lipid bilayer, the $L_m \rightarrow L_a$ phase transition occurring abruptly at $T_m$ involves fundamentally the trans $\rightarrow$ gauche isomerizations, which proceed with rotations of carbon atoms about the C-C single bonds in the acyl chains of the lipid (14). Consequently, the thermally induced phase transition can be discussed in terms of trans $\rightarrow$ gauche isomerizations at $T_m$.

The MM-based molecular model that we have devised originally to describe the $T_m$-lowering effect of acyl chain monounsaturates makes the following three assumptions. 1) The monoenoic PE considered to consist of a long linear segment and a short disordered segment. 2) The short segment does not contribute significantly to the conformational disordering process at $T_m$, since it is already partially disordered at $T_m$. 3) The long linear segment of the sn-2 acyl chain is assumed to undergo a favorable van der Waals contact interaction with the neighboring all-trans sn-1 acyl chain in the gel state bilayer; hence, unlike the short disordered segment, its contribution to the chain melting process at $T_m$ is significant. The strength of the contact interaction, however, depends on the length of the long linear all-trans segment. When the single cis double bond is located near the middle of the sn-2 acyl chain as shown in Fig. 2H. For comparison, the published $T_m$ profiles for monoenoic PE with various positions of the single double bond, $\Delta^\alpha$, are also shown in the inset in Fig. 6.

- Table II

| Lipid          | SS  | $\Delta$C | N  | $T_m^{\text{calc}}$ | $T_m^{\text{obs}}$ | $\Delta T_m$ |
|----------------|-----|-----------|----|---------------------|-------------------|------------|
| C(18):C(18:1)PE | 5   | 3.5       | 33.5 | 5.2                | 5.7               | -0.5       |
| C(18):C(18:2)PE | 5   | 5.5       | 35.5 | 7.9                | 7.2               | -0.6       |
| C(18):C(18:3)PE | 5   | 3.5       | 33.5 | 29.6               | 30.1              | -0.5       |
| C(18):C(18:4)PE | 2   | 5.5       | 35.5 | 32.1               | 31.6              | -0.5       |

The plot of the gel-to-liquid crystalline phase transition temperature ($T_m$) versus the position of the two cis double bonds in the sn-2 acyl chain of C(X):C(20:2$\Delta^{\alpha,n-n}$)PE, where X = 18, 20, and 22, and n = 5, 6, 7, 9, 10, 11, and 14. With the exception of C(20:2$\Delta^{10,13}$)PE, all $T_m$ values are calorimetrically determined values derived from Fig. 4. The $T_m$ values for C(X):C(20:2$\Delta^{10,13}$)PE are calculated values based on Equation 2 and are listed in Table II. In the inset, the experimental $T_m$ values of monoenoic C(X):C(18:1)$\Delta^{\alpha,n-n}$PE is plotted against the position of the single cis double bond, $\Delta^\alpha$, in the sn-2 acyl chain, while $X = 18$ and 20 and $n = 6, 7, 9, 11, 12, 13, and 15$. With the exception of C(X):C(18:1$\Delta^{13}$)PE, all $T_m$ values are calorimetrically determined values taken from the literature (1, 2). The $T_m$ values of C(18):C(18:1$\Delta^{13}$)PE and C(20):C(18:1$\Delta^{13}$)PE are 54.3 and 54.8 °C, respectively, determined recently in this laboratory.

![Fig. 6](image_url)

**Fig. 6.** The plot of the gel-to-liquid crystalline phase transition temperature ($T_m$) versus the position of the two cis double bonds in the sn-2 acyl chain of C(X):C(20:2$\Delta^{\alpha,n-n}$)PE, where X = 18, 20, and 22, and n = 5, 6, 7, 9, 10, 11, and 14. With the exception of C(20:2$\Delta^{10,13}$)PE, all $T_m$ values are calorimetrically determined values derived from Fig. 4. The $T_m$ values for C(X):C(20:2$\Delta^{10,13}$)PE are calculated values based on Equation 2 and are listed in Table II. In the inset, the experimental $T_m$ values of monoenoic C(X):C(18:1)$\Delta^{\alpha,n-n}$PE is plotted against the position of the single cis double bond, $\Delta^\alpha$, in the sn-2 acyl chain, while $X = 18$ and 20 and $n = 6, 7, 9, 11, 12, 13, and 15$. With the exception of C(X):C(18:1$\Delta^{13}$)PE, all $T_m$ values are calorimetrically determined values taken from the literature (1, 2). The $T_m$ values of C(18):C(18:1$\Delta^{13}$)PE and C(20):C(18:1$\Delta^{13}$)PE are 54.3 and 54.8 °C, respectively, determined recently in this laboratory.

All structure parameters have a common unit of C–C bond length.

- Table III

| Lipid          | SS  | $\Delta$C | N  | $T_m^{\text{calc}}$ | $T_m^{\text{obs}}$ | $\Delta T_m$ |
|----------------|-----|-----------|----|---------------------|-------------------|------------|
| C(18):C(18:1)PE | 5   | 3.5       | 33.5 | 5.2                | 5.7               | -0.5       |
| C(18):C(18:2)PE | 5   | 5.5       | 35.5 | 7.9                | 7.2               | -0.6       |
| C(18):C(18:3)PE | 5   | 3.5       | 33.5 | 29.6               | 30.1              | -0.5       |
| C(18):C(18:4)PE | 2   | 5.5       | 35.5 | 32.1               | 31.6              | -0.5       |

The structure parameters (SS, $\Delta C$, and N) and $T_m$ values for various dienoic PE.

- Table IV

| Lipid          | SS  | $\Delta$C | N  | $T_m^{\text{calc}}$ | $T_m^{\text{obs}}$ | $\Delta T_m$ |
|----------------|-----|-----------|----|---------------------|-------------------|------------|
| C(18):C(18:1)PE | 5   | 3.5       | 33.5 | 5.2                | 5.7               | -0.5       |
| C(18):C(18:2)PE | 5   | 5.5       | 35.5 | 7.9                | 7.2               | -0.6       |
| C(18):C(18:3)PE | 5   | 3.5       | 33.5 | 29.6               | 30.1              | -0.5       |
| C(18):C(18:4)PE | 2   | 5.5       | 35.5 | 32.1               | 31.6              | -0.5       |
core. As a result, when \( N \) and \( \Delta C \) are constant, the larger the \( T_m^{\text{cal}} \) becomes (Table II). Hence, the short segment of the kinked \( sn-2 \) acyl chain is basically a perturbation term. The mathematic expression of Equation 2 is thus in complete accord with the second assumption of the MM-based molecular model, implying that the short segment is largely disorder at \( T < T_m \). An important question that needs an answer at this point is, “Are the experimentally observed \( T_m \) values shown in Figs. 3 and 4 consistent with the phase transition process involving primarily the \( sn-1 \) acyl chain and the long linear segment of the \( sn-2 \) acyl chain in the dienoic PE bilayer?” If the answer is yes, then the third assumption of the MM-based molecular model is also applicable to dienoic PE, and hence the model as a whole can be considered as a more general one which can explain the \( T_m \)-lowering effect of acyl chain mono- and di-unsaturation.

The answer to the question just raised does not appear to be a simple one. This is due in part to the possible complication that for dienoic PE the chain melting process of \( trans \rightarrow gauche \) isomerizations at \( T_m \) may involve a fraction of the short segment as its length approaches that of the long segment. Nevertheless, we can test the third assumption by comparing the \( T_m \) of \( C(X):C(Y):2\Delta^{n,n+3} \)PE with that of a set of saturated species of the long \( \text{cis} \) chain, \( C(Y):2\Delta^{n,n+3} \)PE. This selected lipid species may be characterized by a \( Y-3 \) chain length that is equivalent to the length of the long linear all-\( trans \) segment of the kinked \( C(Y):2\Delta^{n,n+3} \) chain. If the third assumption is valid, we can expect that the lipid bilayer composed of \( C(X):C(Y):2\Delta^{n,n+3} \)PE will exhibit calorimetrically the \( L_\beta \rightarrow L_\alpha \) phase transition with a \( T_m \) value that is equivalent to the \( T_m \) associated with the \( L_\beta \rightarrow L_\alpha \) phase transition of the \( C(X):C(Y) \)PE bilayer.

When the two methylene-interrupted \( cis \) double bonds are located mostly in the lower half of the \( sn-2 \) acyl chain, the long linear all-\( trans \) segment of \( C(X):C(Y):2\Delta^{n,n+3} \) chain can be calculated to extend from \( C(3) \) to \( C(n-1) \) with a segment length of \( (n-4) \) carbon-carbon bond lengths (Fig. 2A). This calculation has taken the following two structural features into consideration. 1) The \( sn-2 \) acyl chain bends sharply at \( C(2) \). 2) The \( C(n-1) \) - \( C(n) \) single bond constitutes the initial part of the kink sequence of \( s^\Delta \) s\( ^\Delta \). Let us consider \( C(18):C(18:2\Delta^{n,n+3}) \)PE as an example. The long linear all-\( trans \) segment of its \( sn-2 \) acyl chain contains 6 carbons, extending from \( C(3) \) to \( C(n-1) \) or \( C(n) \) atom. The \( T_m \) value of this unsaturated lipid species can, therefore, be compared directly with that of saturated \( C(18):C(8) \)PE. If, on the other hand, the two methylene-interrupted \( cis \) double bonds are located mostly in the upper half of the \( sn-2 \) acyl chain, the long linear all-\( trans \) segment begins with \( C(n+5) \) atom and ends with the methyl carbon with \( Y-n-5 \) carbon-carbon bond lengths. \( C(18):C(18:2\Delta^{6,9}) \)PE can serve as an example. Its long linear all-\( trans \) segment in the dienoic \( sn-2 \) acyl chain can be shown to extend from \( C(11) \) to \( C(18) \). The corresponding \( C(18):C(Y) \)PE is thus \( C(18):C(10) \)PE with its saturated \( sn-2 \) acyl chain containing an all-\( trans \) segment of 7 C-C bond lengths. It should be mentioned that the lipid bilayer composed of highly asymmetric \( C(18):C(8) \)PE or \( C(18):C(10) \)PE undergoes the mixed-interdigitated (\( L_\alpha \)) phase transition upon heating (15). Nevertheless, the \( T_m \) value associated with the fictive \( L_\alpha \) phase transition for the bilayer composed of highly asymmetric PE can be calculated (12).

For a homologous series of \( C(18):C(18:2\Delta^{n,n+3}) \)PE with \( n \) varying stepwise from 5 to 8, the \( Y \) values of the corresponding \( C(18):C(Y) \)PE can be shown to decrease stepwise from 9 to 6. The \( T_m \) values associated with the fictive \( L_\alpha \) phase transitions for \( C(18):C(Y) \)PE with \( Y=9,8,7, \) and 6 are 29.1, 18.3, 6.0, and \(-8.0\) °C, respectively (12). In contrast, as \( n \) in the dienoic \( C(18:2\Delta^{n,n+3}) \) chain further increases progressively from 9 to 12, the number of carbon atoms in the \( sn-2 \) acyl chain of \( C(18):C(Y) \)PE increases successively from 6 to 9. The \( T_m \) value of the \( C(18):C(Y) \)PE also increases correspondingly with increasing \( n \). These \( T_m \) values of \( C(18):C(Y) \)PE are plotted in Fig. 7A as a function of \( n \) of the corresponding \( C(18):C(18:2\Delta^{n,n+3}) \)PE. Clearly, the connected \( T_m \) curve exhibits the expected V-shaped profile as the two methylene-interrupted \( cis \) double bonds move along the chain as a unit from the \( \Delta^5,8 \) position to the \( \omega 9 \) or \( \Delta^8,12 \) position. Similarly, a calculated \( T_m \) profile can also be derived for \( C(20):C(Y) \)PE from the long linear all-\( trans \) segment of the \( sn-2 \) acyl chain in \( C(20):C(18:2\Delta^{n,n+3}) \)PE. The resulting \( T_m \) curve is nearly superimposable over the one shown in Fig. 7A; hence, it is not illustrated. It should be emphasized that the calculated V-shaped \( T_m \) profile shown in Fig. 7A is the hypothetical \( T_m \) curve for \( C(18):C(18:2\Delta^{n,n+3}) \)PE based on the supposition that only the long all-\( trans \) segment of the \( sn-2 \) acyl chain and the long-\( trans \) \( sn-1 \) acyl
The experimental $T_m$ values listed in Table I for C(X):C(18:2$\Delta^{5,9}$)PE with $X = 18$ and 20 and $n = 6, 9, 12$ are presented in Fig. 7A. It is evident that the experimental $T_m$ values agree well with the corresponding points on the calculated $T_m$ curve for C(X):C(18:2$\Delta^{6,9}$)PE and C(X):C(18:2$\Delta^{12,15}$)PE, suggesting that the third assumption of the MM-based molecular model is indeed valid for these two sets of dienoic PE species. However, the $T_m$ values of C(18):C(18:2$\Delta^{12,15}$)PE and C(20):C(18:2$\Delta^{9,12}$)PE shown in Fig. 7A deviate significantly from the predicted $T_m$ values. These deviations, nonetheless, should not be taken as a surprising result. The long segment of the C(18:2$\Delta^{9,12}$) chain has 6 consecutive C-C single bonds (Fig. 2D); however, its all-trans segment from C(3) to C(8) is only 5 C-C bond lengths long. The short segment from C(13) to C(18) also has 5 single C-C bonds and the short segment. In this case, it is most likely that the entire length of the short segment is not completely disordered at $T < T_m$; hence, it contributes somewhat to the chain melting process at $T_m$, leading to a higher $T_m$ than expected.

The calculated $T_m$ curve and the experimental $T_m$ values for C(X):C(20:2$\Delta^{4,10}$)PE with $X = 18$, 20, and 22 and $n = 5, 8, 11$, and 14 are shown in Fig. 7B. The ω3 or $\Delta^{14,17}$ lipids with highly dynamic short segments are seen to exhibit $T_m$ values that match nearly perfectly with the calculated $T_m$ curve. As the methylene-interrupted two cis double bonds move upward along the chain to the $\Delta^{11,14}$ position, the experimental and calculated $T_m$ values begin to deviate; however, the deviation is not too severe. This moderate deviation can be explained based on the difference in the number of the C-C single bonds between the long all-trans segment and the short segment which is merely 2. As a result, the short segment may provide an additional contribution to the chain melting process of the trans $\rightarrow$ gauche isomerizations, leading to a higher $T_m$. In the case of C(X):C(20:2$\Delta^{8,11}$)PE, the difference in the single C-C bond number between the long linear all-trans segment and the short segment is also 2. However, the short segment is located between C(3) and C(8) without a free end; the methylene units are thus rotationally less dynamic at $T < T_m$. Consequently, this short segment can be expected to have a higher additive contribution to the chain melting process of trans $\rightarrow$ gauche isomerizations at $T_m$. The experimental values of C(X):C(20:2$\Delta^{8,11}$)PE are indeed observed to deviate more from the calculated $T_m$ in comparison with C(X):C(20:2$\Delta^{11,14}$)PE as shown in Fig. 7B.

In the foregoing discussion of the deviation of experimental $T_m$ values from the calculated one, we have introduced a simple concept which can be restated as follows. The short segment of the kinked sn-2 acyl chain in dienoic PE may contribute somewhat to the chain melting process of trans $\rightarrow$ gauche isomerizations at $T_m$ when its length approaches that of the long segment. In principle, this same concept should be equally applicable to describe the $T_m$-lowering phenomenon exhibited by any series of positional isomers of sn-1 saturated/sn-2 monoenoeic PE. With this in mind, we now focus on the calorimetrically determined $T_m$ values for two series of C(18):C(18:1$\Delta^{3}$)PE and C(20):C(18:1$\Delta^{3}$)PE seen in the inset of Fig. 6. These $T_m$ values are replotted in Fig. 7C where a predicted V-shaped $T_m$ profile is also illustrated. When the concept of the contribution of the short segment to $T_m$ is considered, one can expect two characteristic features to be observed for monoenoic PE as follows. 1) The experimental $T_m$ value must deviate increasingly from the predicted one as the single cis double bond moves progressively along the sn-2 acyl chain from either end at $\Delta^{6}$ or $\Delta^{15}$ toward the chain center at $\Delta^{10}$. This is due to the fact that the chain length of the short segment is largest when the single double bond is at $\Delta^{10}$ for sn-2 cis-octadecenoic acyl chain. 2) When the short segments of two positional isomers have the same length, the magnitude of the $T_m$ deviation depends on the location of the short segment. Specifically, the lipid species with the short segment located in the lower half of the sn-2 acyl chain is expected to exhibit a calorimetric $T_m$ value that deviates less from the calculated one. For instance, C(18):C(18:1$\Delta^{3}$)PE and C(18):C(18:1$\Delta^{3}$)PE have a common length of the short segment. When the experimental $T_m$ values of C(18):C(18:1$\Delta^{3}$)PE and C(18):C(18:1$\Delta^{3}$)PE are compared, the former is expected to deviate less from the calculated $T_m$. This expectation is based on the fact that the short segment of the sn-2 acyl chain of C(18):C(18:1$\Delta^{11}$)PE is in the lower half of the lipid molecule with a free methyl end; hence, this short segment is more disordered at $T < T_m$. Consequently, it contributes less to the chain melting process at $T_m$ in comparison with the short segment of its positional isomer of C(18):C(18:1$\Delta^{3}$)PE. A close inspection of Fig. 7C reveals that the two expected features discussed above can indeed be identified, indicating that the fundamentally simple concept of the contribution of the short chain to $T_m$ is supported by our calorimetric results. Based on data shown in Fig. 7, A–C, we can conclude that the simple concept of the contribution of the short chain to $T_m$ does account for the deviation of experimental $T_m$ from the expected one for both mono- and di-unsaturated PE systems.

To sum up, we have determined by DSC the $T_m$ values of aqueous dispersions prepared individually from two subclasses of sn-1 saturated/sn-2 dienoic PE with a total of 18 lipid species. The experimental $T_m$ values are plotted in Fig. 7, A and B, as a function of the positions of the cis double bonds. The calculated $T_m$ curves are also included in Fig. 7, A and B, which are generated based on the third assumption of our previously proposed molecular model (1, 3). Clearly, the experimental and calculated $T_m$ values do not all agree, indicating that our earlier proposed molecular model is not an optimal one and hence it needs to be refined. Here, we offer the following two refinements. 1) Although the all-trans sn-1 acyl chain and the long all-trans segment of the kinked sn-2 acyl chain are assumed to contribute mostly to the chain melting process of trans $\rightarrow$ gauche isomerizations at $T_m$, the short segment may also contribute somewhat to the chain melting process, especially if the length of the short segment approaches that of the long segment. 2) If the positions of the two cis double bonds such as $\Delta^{5,8}$ are located near to the H$_2$O/hydrocarbon interface, the interchain hydration is postulated to increase, leading to a decreased $T_m$. With these refinements, the observed $T_m$ values in Fig. 7, A and B, can be reasonably explained in comparison with the calculated $T_m$ values. Support for the assumption that the short segment may contribute somewhat to the chain melting process at $T_m$ comes from the experimental and calculated $T_m$ values of monoenoic PE as shown in Fig. 7C. Finally, it should be emphasized that the revised molecular model can serve best as a qualitative means to explain the $T_m$-lowering
effect of acyl chain unsaturation at the present time. It remains a challenge to develop a simple and unified mathematical expression in terms of structural parameters in describing precisely the $T_m$ profile observed in the plot of $T_m$ versus $\Delta^{n+3}$ or $\Delta^n$. Nevertheless, the refined MM-based molecular model proposed in this investigation may be further improved in the future to meet the challenge, when the data base is expanded continuously.

REFERENCES
1. Wang, Z., Lin, H., Li, S., and Huang, C. (1994) *J. Biol. Chem.* **269**, 23491–23499
2. Huang, C., Li, S., Lin, H., and Wang, G. (1996) *Arch. Biochem. Biophys.* **334**, 135–142
3. Huang, C., Lin, H., Li, S., and Wang, G. (1997) *J. Biol. Chem.* **272**, 21917–21926
4. Wang, G., Li, S., Lin, H., and Huang, C. (1997) *Biophys. J.* **73**, 283–292
5. Taylor, W. R., and Strong, F. M. (1950) *J. Am. Chem. Soc.* **72**, 4623–4625
6. Christie, W. W., and Holman, R. T. (1967) *Chem. Phys. Lipids* **1**, 407–423
7. Batta, A. K., Dayal, B., Shefer, S., and Salen, G. (1984) *J. Chromatogr.* **284**, 257–260
8. Hitchcock, P. B., Mason, R., Thomas, K. M., and Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 3036–3040
9. Chang, H., and Epand, R. M. (1983) *Biochim. Biophys. Acta* **728**, 319–324
10. Mantsch, H. H., Hsi, S. C., Butler, K. W., and Cameron, D. G. (1983) *Biochim. Biophys. Acta* **728**, 325–330
11. Seddon, J. M., Harlos, K., and Marsh, D. (1983) *J. Biol. Chem.* **258**, 3850–3854
12. Huang, C., Wang, Z., Lin, H., Brumbaugh, E. E., and Li, S. (1994) *Biochim. Biophys. Acta* **1189**, 7–12
13. Keough, K. M. W. (1990) *Biochem. Soc. Trans.* **18**, 835–837
14. Huang, C. (1998) in *The Cell Physiology Source Book* (Sperelakis, N., ed) pp. 39–60, Academic Press, San Diego
15. Mason, J. T. (1996) in *Handbook of Nonmedical Applications of Liposomes* (Lasic, D. D., and Barenholz, Y., eds) Vol. 1, pp. 195–218, CRC Press, Inc., Boca Raton, FL