Effects of Alcohols on the Phase Transition Temperatures of Mixed-Chain Phosphatidylcholines

Shusen Li, Hai-nan Lin, Guoquan Wang, and C. Huang
Department of Biochemistry, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908 USA

ABSTRACT The biphasic effect of ethanol on the main phase transition temperature (T_m) of identical-chain phosphatidylcholines (PCs) in excess H_2O is now well known. This biphasic effect can be attributed to the transformation of the lipid bilayer, induced by high concentrations of ethanol, from the partially interdigitated $L_{\beta'}$ phase to the fully interdigitated $L_{\beta I}$ phase at $T < T_m$. The basic packing unit of the $L_{\beta I}$ phase has been identified recently as a binary mixture of PC/ethanol at the molar ratio of 1:2. The ethanol effect on mixed-chain PCs, however, is not known. We have thus in this study investigated the alcohol effects on the T_m of mixed-chain PCs with different ΔC values, where ΔC is the effective acyl chain length difference between the *sn*-1 and *sn*-2 acyl chains. Initially, molecular mechanics (MM) simulations are employed to calculate the steric energies associated with a homologous series of mixed-chain PCs packed in the partially and the fully interdigitated $L_{\beta I}$ motifs. Based on the energetics, the preference of each mixed-chain PC for packing between these two different motifs can be estimated. Guided by MM results, high-resolution differential scanning calorimetry is subsequently employed to determine the T_m values for aqueous lipid dispersions prepared individually from a series of mixed-chain PCs (ΔC = 0.5–6.5 C-C bond lengths) in the presence of various concentrations of ethanol. Results indicate that aqueous dispersions prepared from mixed-chain PCs with a ΔC value of less than 4 exhibit a biphasic profile in the plot of T_m versus ethanol concentration. In contrast, highly asymmetric PCs ($\Delta C > 4$) do not exhibit such biphasic behavior. In the presence of a longer chain *n*-alcohol, however, aqueous dispersions of highly asymmetric C(12):C(20)PC (ΔC = 6.5) do show such biphasic behavior against ethanol. Our results suggest that the ΔC region in a highly asymmetric PC packed in the $L_{\beta I}$ phase is most likely the binding site for *n*-alcohol.

INTRODUCTION

In 1983, Rowe reported the first observation that ethanol exerts a biphasic effect on the main phase transition temperature (T_m) of fully hydrated phosphatidylcholines (Rowe, 1983). For instance, in the absence of ethanol, the T_m value of the fully hydrated dipalmitoyl-phosphatidylcholine (DPPC) bilayer is 41.6°C. Interestingly, this T_m value decreases linearly with increasing ethanol concentration, and the observed maximum decrease in T_m is about 2°C, which occurs at the ethanol concentration of 1.09 M (or 50 mg/ml). Above this threshold concentration of ethanol (1.09 M), however, the T_m increases with increasing ethanol concentration. The high ethanol effect has been shown subsequently by x-ray diffraction to be due to the transformation of the partially interdigitated $L_{\beta'}$ gel phase into the fully interdigitated $L_{\beta I}$ gel phase in the DPPC bilayer (Simon and McIntosh, 1984). In the fully interdigitated $L_{\beta I}$ gel bilayer, the hydrocarbon thickness and the lateral chain-chain separation distance within the hydrocarbon core of the DPPC bilayer are decreased. Recently it has been shown that above some critical threshold concentrations, short-chain primary and secondary alcohols up to 1-heptanol can induce chain interdigitation in bilayers of dipalmitoyl- and distearoylphosphatidylcholines (DPPCs and DSPCs) (Rowe and Campion, 1994; Löbbecke and Cevc, 1995). Most recently, experimental results from x-ray diffraction studies carried out with bilayers from dimyristoylphosphatidylcholine (DMPC) to DSPC in the presence of short-chain primary alcohols from methanol to 1-butanol have established a structural model for the alcohol-phospholipid complex in the $L_{\beta I}$ gel bilayer. In this alcohol-phospholipid complex, 2 moles of alcohol are identified to interact with 1 mole of lipid molecule. Specifically, each short-chain primary alcohol is positioned at the hydrocarbon/H_2O interface in the interdigitated $L_{\beta I}$ gel bilayer with the hydroxyl group of alcohol standing near the neighboring carbonyl oxygen of a lipid acyl chain and the methyl group of the same alcohol facing the methyl terminus of another lipid acyl chain from the opposing leaflet (Adachi et al., 1995).

All of the alcohol-induced chain interdigitation studies reported in the literature are focused entirely on identical-chain phosphatidylcholines (PCs), in which the effective acyl chain length difference between the *sn*-1 and *sn*-2 acyl chains (ΔC) is constant, being about 1.5 carbon-carbon bond lengths when the PCs are in the gel state bilayer (Huang and Mason, 1986). In an attempt to extend the alcohol effect on identical-chain PC, we have in this communication employed the molecular mechanics (MM) approach to calculate the energetic terms for hydrated mixed-chain PC packed in two different bilayer states in the absence and presence of ethanol or ethanol/*n*-alcohol, where *n*-alcohol is a longer primary alcohol. These energetic terms predict the effects of ethanol or ethanol/*n*-alcohol on chain

Received for publication 5 January 1996 and in final form 29 February 1996.

Address reprint requests to Dr. Ching-hsien Huang, Box 440, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908. Tel.: 804-924-5010; Fax: 804-924-5069.

0006-3495/96/06/2784/11 $2.00

interdigitation in hydrated mixed-chain PC as the ΔC value of mixed-chain PC varies. Here, mixed-chain PC denotes the saturated diacyl PC in which the two acyl chain lengths are different. Specifically, mixed-chain PC is abbreviated as C(X):C(Y)PC, where C(X) preceding the colon refers to the *sn*-1 acyl chain with X carbon atoms, and the notation C(Y) succeeding the colon gives the total number of carbons (Y) in the *sn*-2 acyl chain. The effective chain length difference between the *sn*-1 and *sn*-2 acyl chains in C(X):C(Y)PC in the gel-state bilayer is $\Delta C = |X - Y + 1.5|$ carbon-carbon bond lengths (Huang and Mason, 1986). Guided by MM calculations, high-resolution differential scanning calorimetry (DSC) measurements have also been performed using aqueous dispersions of mixed-chain phospholipids with different ΔC values in the presence of various concentrations of ethanol or ethanol/*n*-alcohol. The DSC results obtained with mixed-chain PC and alcohols are indeed in complete accord with the results predicted by MM calculations.

MATERIALS AND METHODS

Semisynthesis of saturated mixed-chain phosphatidylcholines

A series of mixed-chain PCs was prepared semisynthetically at room temperature using the modified procedure of Mena and Djerassi (1985) as described previously (Lin et al., 1990). This series of mixed-chain PC contains the following five molecular species: C(15):C(17)PC, C(14):C(18)PC, C(17):C(15)PC, C(13):C(19)PC, and C(12):C(20)PC, each of which has the same total number of chain methylene units as that of DPPC. The fatty acids and lysophosphatidylcholines required for the semisynthesis of the above five molecular species of C(X):C(Y)PC were purchased from Sigma (St. Louis, MO) and Avanti Polar Lipids (Alabaster, AL), respectively. Saturated identical-chain diacyl PCs were also obtained from Avanti Polar Lipids. All lipids under study were 99% pure as judged by analytical thin-layer chromatography, as reported earlier (Li et al., 1994). In addition, the positional isomer resulting from mixing of the two acyl chains in each mixed-chain C(X):C(Y)PC is estimated to be less than 2% (Mason et al., 1981).

Preparation of lipid/alcohol samples

Before the lipid sample was prepared, purified PC was lyophilized from benzene. The lyophilized lipid powder (4.0–4.4 mg) was dispersed in 2 ml of aqueous NaCl (50 mM) solution containing 1 mM EDTA and 5 mM phosphate buffer, pH 7.4. This lipid dispersion was sealed under an N_2 atmosphere and heated to a temperature of about 15°C above the T_m value of the lipid species. The sample tube was then subjected to continuous sonication in an ultrasonic water bath for 30–40 min at the elevated temperature. Aliquots (50–250 μl) of absolute ethanol were then added to the buffered lipid dispersion (2 ml) to give the final desired concentration of ethanol in the lipid/ethanol sample. The resealed sample tube was further vortexed thoroughly at room temperature for another 5 min. If a second component of *n*-alcohol was intended to add into the lipid/ethanol dispersion, an aliquot of *n*-alcohol was then added at this step at room temperature before the low-temperature incubation. Otherwise, the ethanol-containing lipid dispersion was thermally equilibrated at 4°C. Unless indicated otherwise, all aqueous samples of lipid/alcohol were incubated at 4°C for a minimum of 24 h before DSC experiments.

If a second *n*-alcohol was to be added into the lipid/ethanol sample, an aliquot of the pure *n*-alcohol was added into the lipid/ethanol dispersion (~2 ml) to give a final molar ratio of 1.2:1.0 *n*-alcohol/lipid. For instance, an aliquot of 0.9 μl of 1-hexanol was added to 2 ml of lipid/ethanol sample containing 4.4 mg of C(12):C(20)PC. In calculating the molar ratio, the density of 1-hexanol was estimated to be 0.8 g/ml.

High-resolution DSC measurements

All aqueous samples of PC/alcohol were studied calorimetrically using a high-resolution MC-2 differential scanning microcalorimeter (Microcal, Northampton, MA) as previously described (Lin et al., 1990). A constant nominal heating scan rate of 15°C/h was generally used. The phase transition temperature T_m of the lipid/alcohol sample was determined from the endothermic peak in the DSC scan using the software provided by Microcal. For each DSC experiment, the reference cell of the calorimeter was filled with the buffered NaCl solution containing the same alcohol concentration as that of the lipid/alcohol sample loaded into the sample cell.

Molecular mechanics calculations

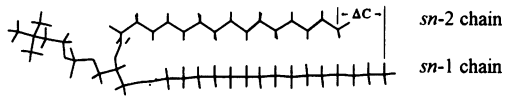
Molecular mechanics (MM) calculations, which include the model building of the energy-minimized structures of monomeric lipid and higher lipid aggregates in the absence of H_2O, or in vacuo, and the generation of the overall steric energy associated with each of the energy-minimized structures, were carried out using Allinger's MM2 (85) program (Allinger, 1977), as described previously (Li et al., 1993). These MM calculations were performed on an IBM RS/6000 computer and Silicon Graphics/Indigo^2 workstations. The molecular graphics representations of various energy-minimized lipid structures arrived at by MM calculations were displayed on a 486 platform using HyperChem Release 4 software package (Hypercube, Waterloo, Canada). Furthermore, HyperChem Release 4 was also used for MM computations if periodic boundary conditions were applied. For instance, lipids, alcohols, and T1P3P water molecules were placed in a three-dimensional periodic box followed by MM^+ energy minimization to obtain the hydration energy associated with the lipid molecule and the lipid/alcohol complex. Computations employing periodic boundary conditions were performed using various three-dimensional boxes; however, the box size cannot exceed the maximum dimensions of 56.104 × 56.104 × 56.104 Å available in the HyperChem software package. For calculating the charge distribution among atoms in the lipid headgroup and the glycerol backbone region, the MNDO method in the HyperChem software package was utilized.

RESULTS

Packing models for aggregated anhydrous phosphatidylcholines

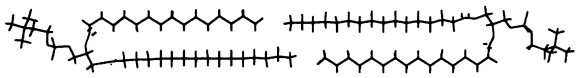
Before modeling the two packing models for aggregated anhydrous phosphatidylcholines, the modified single crystal structure of DMPC (conformation B) was used as the starting structure of monomeric phosphatidylcholine for MM calculations. The modification involved primarily the torsion angle γ_1 of the glycerol moiety CH_2-O bond linked to the *sn*-1 acyl chain. The value of γ_1 was 102° in the original x-ray single crystal work (Pascher et al., 1992) and was modified to 80° here. This modification resulted in an energy-minimized structure of identical-chain monomeric PC with a ΔC value of 2.9 C-C bond lengths (e.g., DPPC, shown graphically in Fig. 1 *A* with a steric energy (E_m) of −40.15 kcal/mol). In this crystalline state, the *sn*-1 and *sn*-2 acyl chains of DPPC are observed to be fully extended; however, the zigzag planes of the two acyl chains are oriented perpendicular to each other (Fig. 1 *A*). Mixed-chain

A. The energy-minimized structure of monomeric DPPC.



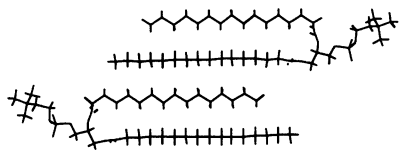
E_m = - 40.15 kcal/mol

B. Trans-bilayer dimer of DPPC packed in the partially interdigitated motif.



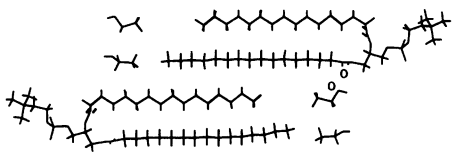
E_d = - 83.40 kcal/mol

C. Fully interdigitated dimer of DPPC.



E_d = - 92.13 kcal/mol

D. Fully interdigitated dimer of DPPC and ethanols packed in the $L_{\beta I}$ state.



E_d = - 94.03 kcal/mol

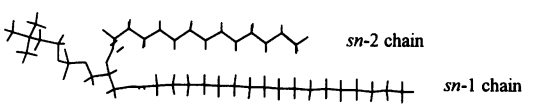
FIGURE 1 Molecular graphics representations of energy-minimized DPPC. (*A*) Monomeric structure and its steric energy (E_m). The effective chain length difference between the *sn*-1 and *sn*-2 acyl chains is denoted by ΔC. In this crystalline structure, ΔC can be seen to be 2.9 C-C bond lengths. (*B*) The trans-bilayer dimer and its steric energy (E_d). (*C*) The fully interdigitated dimer and its steric energy, E_d. (*D*) The fully interdigitated dimer complexed with ethanols as they appeared in the $L_{\beta I}$ phase. Here, monomeric DPPC is noncovalently complexed with two ethanols, whose long molecular axes are aligned along the *sn*-1 and *sn*-2 acyl chains, respectively, of each DPPC molecule. The letter O is used to designate the oxygen atom of one lipid's carbonyl group on the *sn*-1 acyl chain as well as the oxygen atom of the hydroxyl group of an ethanol.

C(*X*):C(*Y*)PCs were modeled similarly in vacuo, based on crystal coordinates of the same modified DMPC molecule using the procedure described earlier (Li et al., 1993; Huang and Li, 1996). The ΔC values, however, were distinctly different for different molecular species of C(*X*):C(*Y*)PC. An example is given in Fig. 2 *A*, which shows the energy-minimized monomeric structure of C(18):C(14)PC, with a ΔC value of 7.0 C-C bond lengths and an E_m value of −38.46 kcal/mol.

Once the energy-minimized structure of a monomeric PC and its steric energy, E_m, were recorded, the next step was to build the structures of dimeric PC based on two packing models: 1) the partially interdigitated packing motif reflecting the packing geometry of two trans-bilayer lipid molecules in the crystalline phase (Li et al., 1993; Huang and Li, 1996), and 2) the fully interdigitated packing motif with and without ethanol, according to the packing motif proposed for the $L_{\beta I}$ bilayer by Adachi et al. (1995), as described in the Introduction. The energy-minimized structures of DPPC packed in these motifs as illustrated by molecular graphics are shown in Fig. 1, *B–D*; the steric energy associated with each energy-minimized dimeric structure (E_d) obtained by the MM2 program is also presented in Fig. 1. The highly asymmetric C(18):C(14)PC obtained from Fig. 2 *A* serves as an example to show the dimeric models (Fig. 2, *B–D*) of mixed-chain PC packed in the partially interdigitated and fully interdigitated motifs. It should be noted from Fig. 2, *B–D*, that the steric energy associated with the energy-minimized dimer (E_d) is different somewhat from the corresponding one calculated for DPPC shown in Fig. 1, *B–D*.

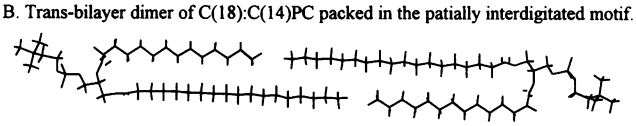
Two types of tetrameric PC were subsequently constructed based on each of the three dimeric structures shown in Fig. 1, *B–D*, and Fig. 2, *B–D*, respectively, by MM

A. The energy-minimized structure of monomeric C(18):C(14)PC.



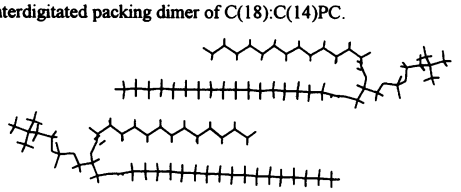
E_m = - 38.46 kcal/mol

B. Trans-bilayer dimer of C(18):C(14)PC packed in the patially interdigitated motif.



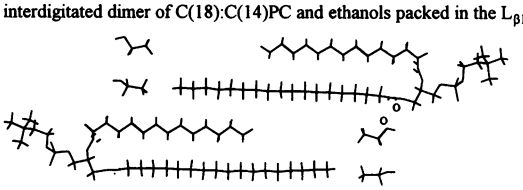
E_d = - 82.62 kcal/mol

C. Fully interdigitated packing dimer of C(18):C(14)PC.



E_d = - 86.76 kcal/mol

D. Fully interdigitated dimer of C(18):C(14)PC and ethanols packed in the $L_{\beta I}$ state.



E_d = - 88.40 kcal/mol

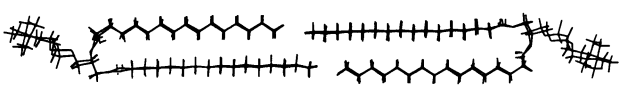
FIGURE 2 Molecular graphics representations of the energy-minimized structure(s) of highly asymmetric C(18):C(14)PC. (*A*) The monomer and its steric energy, E_m. (*B*) The trans-bilayer dimer with its steric energy, E_d, which differs from the corresponding value calculated for trans-bilayer dimer of DPPC, shown in Fig. 1 *B*, by only 0.78 kcal/mol. (*C*) The fully interdigitated dimer and the dimer's steric energy, E_d. (*D*) The fully interdigitated dimer complexed with ethanols. The steric energy, −88.40 kcal/mol, of this complex is considerably greater than the corresponding one, −94.03 kcal/mol, calculated for the DPPC/ethanol complex shown in Fig. 1 *D*, suggesting that the highly asymmetric C(18):C(14)PCs are less likely to associate into the $L_{\beta I}$ phase in the presence of ethanol.

simulations. In addition, the same two types of tetrameric C(14):C(18)PC, C(13):C(19)PC, and C(12):C(20)PC, were also constructed based on the partially interdigitated motif and the fully interdigitated motif in the presence of ethanol. These two types of tetramers were the front-to-back (F-B) tetramer and the up-and-down (U-D) tetramer (Li et al., 1993; Li and Huang, 1996). As an example, the F-B and U-D tetramers for DPPC packed in the partially interdigitated motif, after energy minimization, are illustrated graphically in Fig. 3. The averaged sum of these two types of tetramers was taken to mimic, to a first approximation, the orthorhombic closed-packed structure of PC in the bilayer plane, as described previously (Li et al., 1993; Wang et al., 1995).

The steric energies associated with the two types of tetramers constructed on the basis of a given packing motif for various C(X):C(Y)PC can be obtained by MM calculations. The steric energies of the tetramers with the packing geometry of the partial interdigitation (tp), the full interdigitation with alcohol (tf), and the full interdigitation without ethanol (tf′) are abbreviated as $E_{tp}^{\text{F-B (or UD)}}$, $E_{tf}^{\text{F-B (or U-D)}}$, and $E_{tf'}^{\text{F-B (or U-D)}}$, respectively, where the superscripts F-B and U-D denote the F-B and U-D packing motifs, respectively. The values of $E_{tp}^{\text{F-B}}$ and $E_{tp}^{\text{U-D}}$ for DPPC are given in Fig. 3; other values of $E_{tf}^{\text{F-B (U-D)}}$ and $E_{tf'}^{\text{F-B (U-D)}}$ are −278.64 (−201.30) and −265.15 (−196.01) kcal/mol, respectively. The affinity of four monomers for each other within the aggregated tetramer is a measure of the averaged stability of the tetramer contributed by its constituent monomers. This stability can be expressed as follows: $\Delta E_t = (E_t - 4E_m)/4$. For instance, the magnitudes of $\Delta E_{tp}^{\text{F-B}}$ and $\Delta E_{tp}^{\text{U-D}}$, given in Fig. 3 for DPPC, are −18.96 and −14.07 kcal/mol, respectively. Here, the partially interdigitated tetrameric DPPC with a F-B packing motif is more stable than that with a U-D motif due to its smaller $\Delta E_{tp}^{\text{F-B}}$ value of −18.96 kcal/mol. When the two types of tetramer are closely packed to mimic an orthorhombic two-dimensional lattice, then the overall averaged stabilization energy, ΔE_{tp}^{ave}, is 1/2 $(\Delta E_{tp}^{\text{F-B}} + \Delta E_{tp}^{\text{U-D}}) = -16.52$ kcal/mol (Fig. 3). Similarly, the calculated ΔE_{tf}^{ave} and $\Delta E_{tf'}^{ave}$ values are −19.97 and −17.49 kcal/mol, respectively. Without hydration, the bilayer model for DPPC with a fully interdigitated packing motif, in the presence of ethanol, packed in an orthorhombic lattice has the most stable structure, as indicated by the smallest value of ΔE_{tf}^{ave}. This can be easily recognized as being due to the extensive chain-chain interactions and the additional lateral interactions derived from the added ethanols. The ΔE^{ave} values for energy-minimized tetramers of C(14):C(18)PC, C(13):C(19)PC, C(12):C(20)PC, and C(18):C(14)PC with the partially interdigitated and the fully interdigitated $L_{\beta I}$ motifs packed in the orthorhombic subcell have also been calculated as summarized in Table 1.

For a highly asymmetric C(18):C(14)PC with a ΔC value of 7.0, the ternary complexes of lipid/n-alcohol/ethanol (1:1:1) packed in the fully interdigitated mode were also simulated using Allinger's MM2 program, where n-alcohol

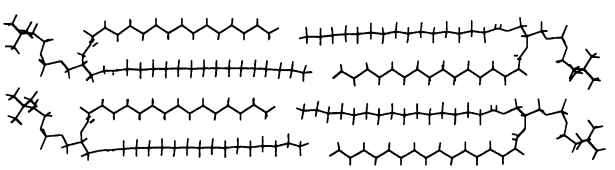
A. The F-B tetramer of DPPC packed in the partially interdigitated motif.



$E_{tp}^{F\text{-}B} = -236.46$ kcal/mol

$\Delta E_{tp}^{F\text{-}B} = (E_{tp}^{F\text{-}B} - 4E_m)/4 = -18.96$ kcal/mol

B. The U-D tetramer of DPPC packed in the partially interdigitated motif.



$E_{tp}^{U\text{-}D} = -216.88$ kcal/mol

$\Delta E_{tp}^{U\text{-}D} = (E_{tp}^{U\text{-}D} - 4E_m)/4 = -14.07$ kcal/mol

$\Delta E_{tp}^{ave} = (\Delta E_{tp}^{F\text{-}B} + \Delta E_{tp}^{U\text{-}D})/2 = -16.52$ kcal/mol

FIGURE 3 Energy-minimized structure of the dimer of trans-bilayer dimeric DPPC. (*A*) The tetramer with an F-B motif. It is characterized by the superposition of two trans-bilayer dimers stacked in an eclipsed position. The steric energy is −236.46 kcal/mol. The stabilization energy of association of the tetramer contributed by each of its four monomers is −18.96 kcal/mol. (*B*) The tetramer with a U-D motif. Here, two trans-bilayer dimers are aligned side by side, so that they are coplanar. The steric energy of this tetramer and the stabilization energy of association contributed by each of its monomers are −216.88 and −14.07 kcal/mol, respectively. The overall averaged stabilization, ΔE_{tp}^{ave}, for the two motifs is −16.52 kcal/mol.

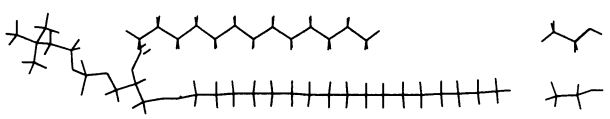
TABLE 1 Various energetic terms, calculated for model bilayers composed of different lipids packed in the partially interdigitated motif or the fully interdigitated $L_{\beta I}$ motif with ethanols

Lipid	ΔC*	$-\Delta E^{ave}$	$-E_{hyd}$	$-E^{total}$	ΔE_{dif}^{total}
DPPC, p	2.94	16.52	15.24	31.76	—
DPPC, f	2.94	19.97	12.71	32.68	−0.92
C(14):C(18)PC, p	1.05	15.99	15.24	31.23	—
C(14):C(18)PC, f	1.05	20.89	12.71	33.60	−2.37
C(13):C(19)PC, p	3.08	18.40	15.24	33.64	—
C(13):C(19)PC, f	3.08	21.38	12.71	34.09	−0.45
C(12):C(20)PC, p	5.05	18.55	15.24	33.79	—
C(12):C(20)PC, f	5.05	20.55	12.71	33.26	0.53
C(18):C(14)PC, p	7.00	17.89	15.24	33.13	—
C(18):C(14)PC, f	7.00	18.60	12.71	31.31	1.82
C(18):C(14)PC, fb	7.00	20.30	12.71	33.01	0.12
C(18):C(14)PC, fh	7.00	21.67	12.71	34.38	−1.25
C(18):C(14)PC, fo	7.00	23.85	12.71	36.56	−3.43

For C(18):C(14)PC bilayers, various energies based on (1:1:1) ternary complex of lipid/ethanol/1-butanol (fb), lipid/ethanol/1-hexanol (fh) or lipid/ethanol/1-octanol (fo) packed in the $L_{\beta I}$ motif are also calculated. Values are in kcal/mol. p, partially interdigitated; f, fully interdigitated.
*ΔC is the effective chain length difference between the *sn*-1 and *sn*-2 acyl chains of PC packed in the crystalline state. ΔE^{ave} is the overall averaged stabilization energy and is illustrated in Fig. 3. E_{hyd} is the hydration energy as illustrated in Fig. 5. E^{total} is the sum of ΔE^{ave} and E_{hyd}. Finally, $\Delta E_{dif}^{total} = E_f^{total} - E_p^{total}$.

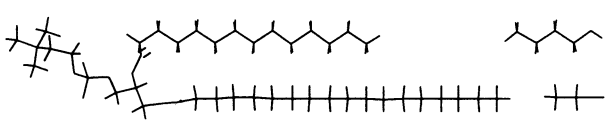
was one of the following primary alcohols: 1-butanol, 1-hexanol, and 1-octanol. Before energy minimization, each ternary complex was modeled according to the following packing specificities. The chain axis of the longer all-*trans* *sn*-1 acyl chain of C(18):C(14)PC was positioned in line with the molecular axis of ethanol, with a separation distance of 4.0 Å between the two methyl ends. Similarly, the shorter *sn*-2 acyl chain of the same C(18):C(14)PC molecule was aligned with the longer *n*-alcohol; however, the hydroxyl group of the longer *n*-alcohol was packed laterally against the hydroxyl group of the neighboring ethanol molecule. In this packing mode, the separation distance between the methyl end of the *sn*-2 acyl chain of C(18):C(14)PC and the methyl end of the *n*-alcohol decreases with increasing number of carbon atoms in *n*-alcohol. The energy-mimimized structures of these ternary complexes are illustrated by molecular graphics in Fig. 4. Here the longer *n*-alcohol and the shorter ethanol are facing the shorter *sn*-2 and longer *sn*-1 acyl chains of C(18):C(14)PC, respectively. It should be emphasized that the linear hydrocarbon chain of *n*-alcohol is located in a hydrophobic pocket generated by the difference in the *sn*-1 and *sn*-2 acyl chain lengths (the ΔC region), as illustrated in Fig. 4. Furthermore, the various steric energy terms, including the overall averaged stabilization energy (ΔE_{tf}^{ave}), are also given in Fig. 4 and Table 1.

A. One C(18):C(14)PC complexed with two ethanols.



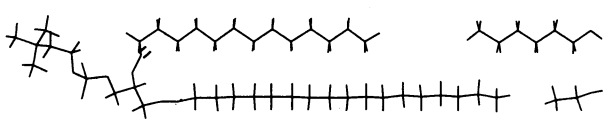
E_m= -38.15 kcal/mol ΔE_{tf}^{ave} = -18.60 kcal/mol

B. One C(18):C(14)PC complexed with one 1-butanol and one ethanol.



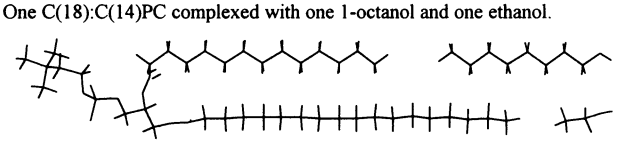
E_m= - 39.70 kcal/mol ΔE_{tf}^{ave} = - 20.31 kcal/mol

C. One C(18):C(14)PC complexed with one 1-hexanol and one ethanol.



E_m= - 39.03 kcal/mol ΔE_{tf}^{ave} = - 21.67 kcal/mol

D. One C(18):C(14)PC complexed with one 1-octanol and one ethanol.



E_m= - 39.56 kcal/mol ΔE_{tf}^{ave} = - 23.85 kcal/mol

FIGURE 4 The simulated structures of C(18):C(14)PC complexed with (*A*) two ethanols, (*B*) one butanol and one ethanol, (*C*) one 1-hexanol and one ethanol, (*D*) one 1-octanol and one ethanol. The values of the steric energy and the overall averaged stabilization energy, ΔE_{tf}^{ave} = ½ ($E_{tf}^{F\text{-}B}$ + $E_{tf}^{U\text{-}D}$), are also given for each binary or ternary complex.

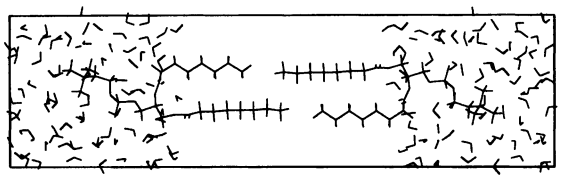
Hydrated structures and hydration energies

Water is a polar molecule, the hydrogen atoms of which are partially positive and whose oxygen atom is partially negative. The headgroup of a phosphatidylcholine molecule is zwitterionic, with a positive charge delocalized among the various atoms in the -$N(CH_3)_3$ group and a negative charge distributed around the phosphate group. In addition, the primary and secondary ester groups are also partially charged at the glycerol backbone region in a PC molecule. Consequently, when the partially interdigitated PC bilayer is immersed in an aqueous medium, H_2O molecules can interact electrostatically with the lipid headgroups on the bilayer's two surfaces; moreover, H_2O molecules can also penetrate into the two glycerol backbone regions and interact with the partially negative carbonyl oxygens. These electrostatic interactions will result in a favorable interaction energy in addition to other energies existing in the anhydrous PC bilayer, thus giving rise to a more stabilized bilayer structure in water. This additional energy, which may be conveniently denoted as the hydration energy (E_{hyd}), can be estimated by MM calculations using the HyperChem software as described below.

Let us begin the estimation of E_{hyd} with a trans-bilayer dimeric PC such as C(8):C(8)PC packed in the partially interdigitated motif followed by that of the fully interdigitated DPPC. First, the energy-minimized structure and the steric energy of a dimeric C(8):C(8)PC, in the presence of H_2O molecules, are generated by HyperChem's MM^+ program, employing the periodic boundary conditions in a three-dimensional rectangular box (Fig. 5 *A*). In arriving at the results, the following assumptions have been made: 1) The lipid headgroups are considered to be immobilized. For PC in the gel or crystalline state, this assumption, to a first approximation, is valid. 2) Before energy minimization, H_2O molecules are randomly placed on the two surfaces of the trans-bilayer dimer and in the glycerol backbone regions up to the level near the outer edges of each carbonyl oxygen of the *sn*-1 acyl chain. Because H_2O can undergo fast exchange, at $T < T_m$, near bilayer surfaces, the assumed random structure of H_2O molecule is thus not unreasonable. 3) The rectangular box size is 14.71 × 13.53 × 51.57 Å. This choice is based on a closest lateral chain-chain distance between two lipids of about 4.8 Å (Simon and McIntosh, 1984) and the assumption that within the interstitial space between two bilayers, water can extend about 5 Å away from the methyl end of the -$N(CH_3)_3$ group in the lipid headgroup. Within this rectangular box, 154 molecules of H_2O are present, as shown in Fig. 5 *A*.

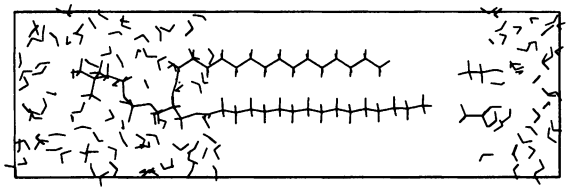
The hydrated dimeric C(8):C(8)PC in the rectangular box can be energy-minimized using HyperChem's MM^+ pro-

A. The hydration box for trans-bilayer dimeric C(8):C(8)PC :



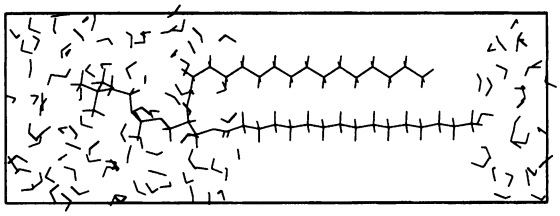
E_s^h = - 236.33 kcal/mol; E_s^a = 97.91 kcal/mol; E_s^w = - 303.76 kcal/mol; E_{hyd} = - 15.24 kcal/mol

B. The hydration box for DPPC/ethanol (1:2) complex :



E_s^h = - 218.54 kcal/mol; E_s^a = 59.53 kcal/mol; E_s^w = - 252.64 kcal/mol; E_{hyd} = -12.71 kcal/mol

C. The hydration box for DPPC without ethanol :



E_s^h = -181.79 kcal/mol; E_s^a = 59.66 kcal/mol; E_s^w = - 224.48 kcal/mol; E_{hyd} = - 8.48 kcal/mol

FIGURE 5 The hydration box for (*A*) the trans-bilayer dimeric C(8):C(8)PC, (*B*) the binary mixture of DPPC/ethanol (1:2), and (*C*) monomeric DPPC. The various energy terms are defined in the text. These boxes are generated from the HyperChem's software package.

gram to yield a steric energy (E_s^h) of −236.33 kcal/mol (Fig. 5 *A*). Within the same periodic box-confined energy-minimized system, the steric energy of the anhydrous dimeric C(8):C(8)PC as well as the 154 H_2O molecules, E_s^a and E_s^w, can also be calculated separately using the MM^+ program, yielding 97.91 and −303.76 kcal/mol, respectively. The hydration energy, E_{hyd}, per monomeric PC per unit hydration surface can thus be shown to be $E_{hyd} = [E_s^h - (E_s^a + E_s^w)]/2 = -15.24$ kcal/mol. Here, the unit hydration surface corresponds to the sum of the cross-sectional areas of the two acyl chains located on the headgroup side of each monomeric PC surface.

The hydrated monomeric DPPC with two molecules of ethanol packed in the fully interdigitated $L_{\beta I}$ state is shown in Fig. 5 *B*. Here, two hydration surfaces per DPPC/ethanol complex (1:2) are observed. The rectangular box has the dimensions 14.91 × 12.98 × 48.02 Å and contains 127 H_2O molecules. In this case, H_2O molecules are allowed to enter the interfacial region of DPPC near the inner edge of the carbonyl oxygen due to the presence of the hydroxyl group of ethanol in the immediate neighborhood. The steric energies of the hydrated DPPC/ethanol, anhydrous DPPC/ethanol, and 127 H_2O molecules in the same box-confined energy-minimized configuration, shown in Fig. 5 *B*, are −218.54, 59.53, and −252.64 kcal/mol, respectively, yielding a hydration energy (E_{hyd}) per monomer PC per unit hydration surface of −12.71 kcal/mol. Similarly, based on the energy-minimized system of hydrated DPPC without ethanol in a three-dimensional box of 14.91 × 12.60 × 41.85 Å containing 114 H_2O molecules (Fig. 5 *C*), the steric energies of the hydrated DPPC, anhydrous DPPC, and 114 H_2O molecules can be calculated separately by HyperChem's MM^+ program to be −181.79, 59.66, and −224.48 kcal/mol, respectively, yielding a hydration energy (E_{hyd}) per monomeric PC per hydration surface of −8.48 kcal/mol. The various values of the calculated E_{hyd} are summarized in Table 1.

In deriving the E_{hyd} term, it is intrinsically assumed that H_2O molecules are virtually totally excluded from the bulky hydrocarbon chain region of the lipid and the region occupied by the CH_3CH_2- moiety of ethanol (Fig. 5, *A–C*). Consequently, the value of E_{hyd} is invariant for PC with different values of ΔC or chain lengths, provided that these different PCs are assembled in the same partially or fully interdigitated motif. In the presence of primary alcohol, the value of E_{hyd} is similarly invariant, irrespective of the kind of alcohol. For instance, the value of E_{hyd} (−15.24 kcal/mol) calculated for C(8):(8)PC packed in the partially interdigitated motif is taken to be the same E_{hyd} value for DPPC packed in the partially interdigitated motif; likewise, the value of −12.71 kcal/mol calculated for DPPC packed in the fully interdigitated motif in the presence of ethanol is taken to be equal to the E_{hyd} value for C(18):C(14)PC packed in the $L_{\beta I}$ state in the presence of ethanol or 1-butanol. Most importantly, this hydration term can, as a first approximation, be added to the averaged stabilization energy (ΔE^{ave}) derived from various anhydrous lipids with different ΔC values to give the total stabilization energy (E^{total}) for hydrated aggregated PC. The calculated values of the total stabilization energy (E^{total}) for various hydrated PC molecules packed in two different motifs are presented in Table 1.

The relationship between the ethanol-induced full interdigitation and the effective chain length difference as predicted by MM modeling

The magnitude of the effective chain length difference between the *sn*-1 and *sn*-2 acyl chains (ΔC) of a PC molecule packed in the crystalline or gel-state lipid assembly reflects the overall asymmetric geometry of that lipid molecule. This geometry can be considered an important factor contributing to the stability of the lipid assembly as a whole. For instance, if the geometry of a PC monomer specified by its ΔC value is such that extensive and favorable interactions can occur among the monomers within the lipid assembly characterized by the partially interdigitated motif, then this PC tends to associate favorably into the lipid assembly with a partially interdigitated packing motif. Fur-

thermore, the affinity of monomeric PCs for each other within a bilayer, which is approximated in this study by the sum of two F-B and U-D tetramers with orthorhombic two-dimensional close packing, is expressed quantitatively by the averaged stabilization energy or ΔE^{ave} (Li et al., 1993; Wang et al., 1995). In water, this averaged stabilization energy is enhanced by the hydration energy (E_{hyd}), resulting in a total stabilization energy (E^{total}) as follows: $E^{total} = \Delta E^{ave} + E_{hyd}$. The magnitudes of E^{total} for crystalline DPPC packed in the partially interdigitated motif and the fully interdigitated motif with and without ethanol can thus be calculated to be −31.76, −32.68, and −25.97 kcal/mol, respectively. Based on the relative magnitude of E^{total}, it is evident that DPPC packed in the partially interdigitated motif is much more stable than DPPC packed in the fully interdigitated motif without ethanol (−31.76 versus −25.97 kcal/mol). In the presence of ethanol, however, the fully interdigitated DPPC/ethanol complex (1:2) is slightly more stable than the partially interdigitated DPPC bilayer (−32.68 versus −31.76 kcal/mol).

Now we can examine the lipid bilayer composed of a specific PC species with a fixed ΔC value; in particular, we can determine whether this PC bilayer can be expected to undergo the ethanol-induced partially interdigitated → $L_{\beta I}$ phase transition. Specifically, the stability of the PC bilayer between these two states (or packing motifs) will be compared by taking the difference between their total stabilization energies as follows: $\Delta E^{total}_{dif} = E^{total}_{f} - E^{total}_{p}$, where the subscripts f and p represent the fully interdigitated motif with ethanol and the partially interdigitated motif without ethanol, respectively, and the subscript dif refers to the difference between the two states. If the value of ΔE^{total}_{dif} is positive, then the bilayer with a partially interdigitated motif is more stable; otherwise, the bilayer with ethanol characterized by a fully interdigitated $L_{\beta I}$ motif is more stable. Hence, lamellar PC with a negative ΔE^{total}_{dif} value can be expected to undergo the ethanol-induced partially interdigitated → $L_{\beta I}$ phase transition. The ΔE^{total}_{dif} values for various PC and PC/ethanol complexes obtained by MM modeling are given in Table 1. Fig. 6 *A* shows the plot of ΔE^{total}_{dif} versus ΔC for a homologous series of five molecular species of PC and their complexes with ethanol. A least-squares line with a positive slope and a correlation coefficient of 0.9921 is obtained. Clearly, PCs with $\Delta C < 4.2$ have negative ΔE^{total}_{dif} values, indicating that these PCs can be expected to undergo the ethanol-induced partially interdigitated → fully interdigitated phase transition. In contrast, highly asymmetric PCs ($\Delta C > 4.2$) are not expected to undergo the ethanol-induced partially interdigitated → $L_{\beta I}$ phase transition, because their ΔE^{total}_{dif} values are positive.

Based on the plot shown in Fig. 6 *A*, the lipid bilayer composed of highly asymmetric C(18):C(14)PC with a ΔC value of 7.0 as depicted in Fig. 2 *A* is not expected to undergo the ethanol-induced partially interdigitated → $L_{\beta I}$ phase transition. This immediately raises an interesting question of whether any of the longer *n*-alcohols together with ethanol can induce chain interdigitation in highly asymmetric C(18):C(14)PC. The values of ΔE^{total}_{dif} for various C(18):C(14)PC/*n*-alcohol/ethanol (1:1:1) ternary complexes, obtained by MM$^+$ calculations confined in hydrated boxes, are given in Table 1. When these ΔE^{total}_{dif} values are plotted against the total carbon number in *n*-alcohol (N_c) as depicted in Fig. 6 *B*, a least-squares line with a negative slope and a correlation coefficient of 0.9958 is obtained. This least-squares line intersects the horizontal line of $\Delta E^{total}_{dif} = 0$ at N_c of 4.2. Clearly, values of ΔE^{total}_{dif} are negative for the two ternary complexes of C(18):C(14)PC/1-hexanol/ethanol (1:1:1) and C(18):C(14)PC/1-octanol/ethanol (1:1:1); results of our MM simulations thus predict that the highly asymmetric C(18):C(14)PC in the bilayer at $T < T_m$ can be induced by 1-hexanol/ethanol or 1-octanol/ethanol to undergo the partially interdigitate → $L_{\beta I}$ phase transition.

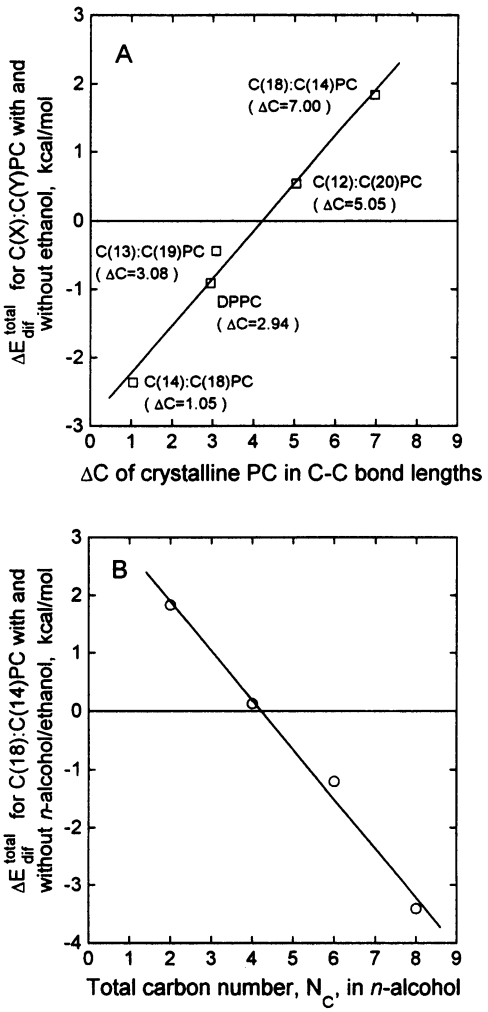


FIGURE 6 (*A*) The total stability energy difference of the mixed-chain PC bilayer is plotted against the ΔC value. If the value of ΔE^{total}_{dif} is negative for a given C(X):C(Y)PC specified by its ΔC, this mixed-chain PC bilayer can be expected to undergo the partially interdigitated → $L_{\beta I}$ phase transition. (*B*) The total stabilization energy difference between the C(18):C(14)PC bilayer and the bilayer composed of a ternary complex of C(18):C(14)PC/*n*-alcohol/ethanol is plotted against the total number of carbon atoms, N_c, in *n*-alcohol. The various *n*-alcohols are indicated in Fig. 4, and the total stabilization energy difference is defined in Table 1.

DSC studies of C(*X*):C(*Y*)PC in the presence of various concentrations of ethanol

Guided by MM results shown in Fig. 6 *A*, high-resolution differential scanning calorimetry was employed to examine the ethanol effect on the T_m of lipid dispersions prepared individually from a homologous series of C(*X*):C(*Y*)PC, each of which has the same MW as that of DPPC. These C(*X*):C(*Y*)PC are C(15):C(17)PC, C(14):C(18)PC, C(17):C(15)PC, C(13):C(19)PC, and C(12):C(20)PC with ΔC values, in the gel state, of 0.5, 2.5, 3.5, 4.5, and 6.5 C-C bond lengths, respectively. To demonstrate first that the procedure described in Materials and Methods for the sample preparation was viable, the DSC experiments were first carried out with DPPC/ethanol samples at various ethanol concentrations ranging from 0 to 120 mg/ml. The biphasic effect of ethanol on the T_m of DPPC liposomes was indeed observed; moreover, the ethanol threshold concentration of 50 mg/ml was also observed (data not shown). These results were thus in total agreement with the published DSC data (Ohki et al., 1990; Roth and Chen, 1991), indicating the validity of our experimental procedure. To further ascertain that the lipid and ethanol in the aqueous sample were at equilibrium, the lipid samples containing a fixed ethanol concentration (40 mg/ml) were scanned calorimetrically after incubating at 4°C for various time intervals. As shown in Fig. 7, DSC results clearly indicate that the same T_m value is exhibited by virtually all samples, each of which has been incubated at 4°C for 24 h or longer. Hence, the method that we had employed for preparing the lipid/alcohol mixture was indeed a valid one.

In Fig. 8, the T_m values of various ethanol-containing lipid dispersions are plotted against the ethanol concentration. It is evident that for C(15):C(17)PC, C(14):C(18)PC, and C(17):C(15)PC, the T_m values decrease initially with increasing concentration of ethanol; however, after the threshold concentration, the T_m values increase with increasing ethanol concentration. Here, the threshold concentration is defined as the ethanol concentration corresponding to the point of minimum T_m in each biphasic curve. The threshold concentrations for C(15):C(17)PC, C(14):C(18)PC, and C(17):C(15)PC, determined by the least-squares polynomial fit, are 50, 63, and 65 mg/ml, respectively. For C(13):C(19)PC and C(12):C(20)PC, two linear lines with negative slopes are observed in the T_m versus ethanol concentration plot (Fig. 8). It should be remembered that the ΔC values for gel-state C(17):C(15)PC and C(13):C(19)PC are 3.5 and 4.5, respectively. Our DSC results shown in Fig. 8 thus indicate that ethanol exerts a biphasic effect on the T_m of the lipid bilayer composed of C(*X*):C(*Y*)PC, which has a ΔC value smaller than 4.0; moreover, the biphasic effect of ethanol is abolished completely for the highly asymmetric C(*X*):C(*Y*)PC with a ΔC value greater than 4.0. Based on MM calculations and the ΔC values for crystalline C(*X*):C(*Y*)PC, it has been predicted that the C(*X*):C(*Y*)PC bilayer can undergo the ethanol-induced partially interdigitated $\rightarrow L_{\beta I}$ phase transition, when the value of crystalline ΔC is less than 4.2 (Fig. 6 *A*). This prediction is indeed verified by DSC results, provided that the biphasic curve observed in Fig. 8 reflects the partially interdigitated $\rightarrow L_{\beta I}$ phase transition which, of course, is well known for the DPPC bilayer (Simon and McIntosh, 1984). Furthermore, the ΔC values shown in Figs. 6 *A* and 8 represent the effective chain length difference between the *sn*-1 and *sn*-2 acyl chains for crystalline and gel-state PC, respectively. Nevertheless, data presented in Fig. 8 show, for the first time, that ethanol can exert a biphasic effect on the T_m exhibited by mixed-chain PC bilayers, and the biphasic effect of ethanol depends critically on the ΔC value of the mixed-chain PC. It should also be said that in solution the mixed-chain lipid and ethanol are in dynamic equilibrium. At the threshold concentration, only a fraction of the mixed-chain PCs are associated transiently with ethanol molecules to form the binary mixture with a molar ratio of 1 to 2. Phrased differently, not all lipids at the threshold concentration are packed in the $L_{\beta I}$ phase. However, the concentration of the 1:2 complex increases with increasing concentration of ethanol, resulting in an up-shift in T_m, as shown in Fig. 8.

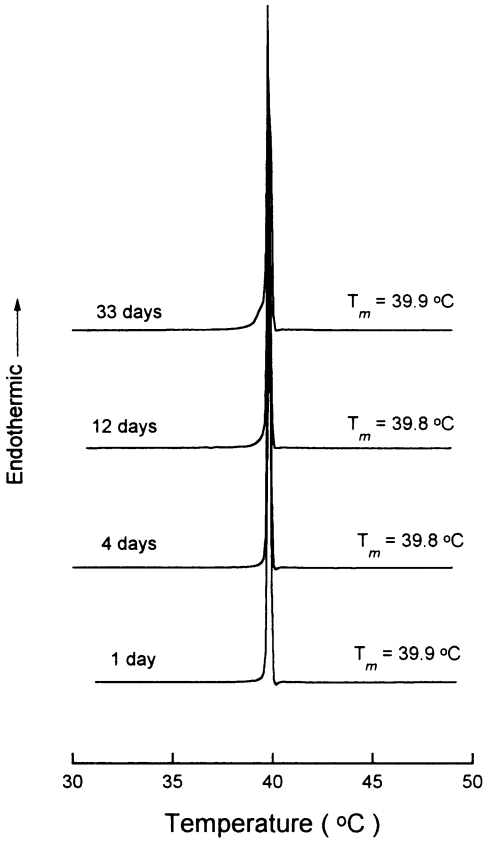


FIGURE 7 The heating DSC scans of aqueous lipid dispersions containing the same fixed amounts of DPPC (2 mg/ml) and ethanol (40 mg/ml). Before DSC scans, these dispersions have been incubated at 4°C for various time intervals as indicated.

DSC studies of C(12):C(20)PC/*n*-alcohol (molar ratio 1:1.2) in the presence of various concentrations of ethanol

Of the five molecular species employed in DSC experiments shown in Fig. 8, C(12):C(20)PC is the most asym-

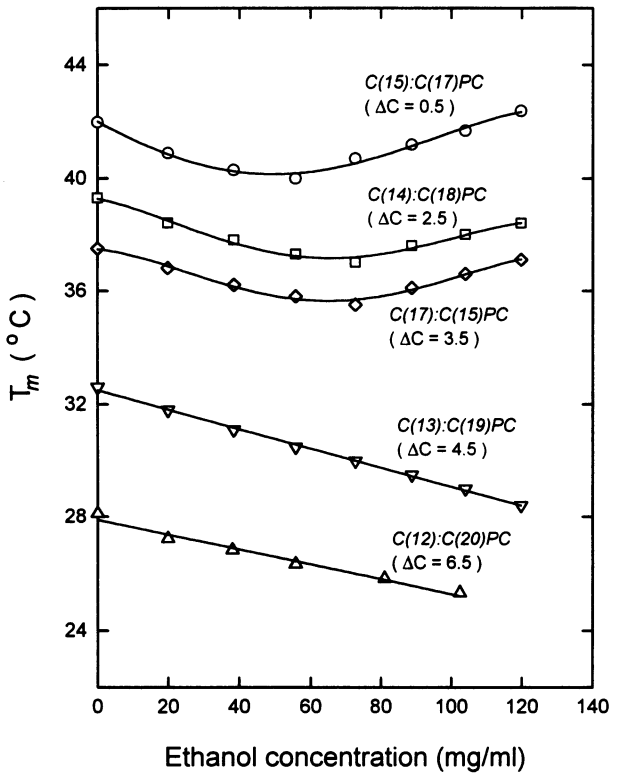


FIGURE 8 The plot of T_m versus ethanol concentration. Five different C(X):C(Y)PCs were studied. Each lipid species was mixed with different concentrations of ethanol. The ethanol-containing lipid dispersion, after incubating at 4°C for a minimum of 24 h, was subjected to DSC scan. The T_m value was obtained form the DSC curve as described in the text. Each ΔC value, in C-C bond lengths, under the appropriate lipid is calculated from the relation: $\Delta C = |X - Y + 1.5|$ for a C(X):C(Y)PC molecule packed in the gel-state bilayer.

metric one, with a ΔC value, in the gel state, of 6.5 C-C bond lengths. According to the calculated results of MM modeling shown in Fig. 6 *B*, highly asymmetric C(18):C(14)PC with a crystalline ΔC value of 7.0 is energetically favorable to complex with 1-hexanol (or 1-octanol)/ethanol in the $L_{\beta I}$ phase; hence, highly asymmetric PC/1-hexanol (or 1-octanol) complex (1:1) can be induced by ethanol to undergo the partially interdigitated → fully interdigitated $L_{\beta I}$ phase transition. Guided by the computational data shown in Fig. 6 *B*, DSC experiments were carried out with complexes of C(12):C(20)PC/1-hexanol (or 1-octanol) (1:1.2 molar ratio) as a function of ethanol concentrations. The T_m values of the *n*-alcohol containing C(12):C(20)PC dispersions are illustrated in Fig. 9 as a function of ethanol concentration. Here, *n*-alcohol represents 1-butanol, 1-hexanol, or 1-octanol. It is evident that highly asymmetric C(12):C(20)PC complexed with 1-butanol does not exhibit the biphasic T_m profile in Fig. 9, suggesting that C(12):C(20)PC bilayers in the presence of 1-butanol do not undergo the ethanol-induced partially interdigitated → fully interdigitated $L_{\beta I}$ phase transition. This is indeed predicted by our MM results, as shown in Fig. 6 *B*.

For aqueous dispersions of C(12):C(20)PC in the presence of a fixed concentration of 1-hexanol, the T_m value is observed to decrease sharply by about 11.4°C at low concentrations of ethanol (20 mg/ml). Above 20 and up to 80 mg/ml of ethanol, however, the transition temperature is concave up (*middle curve* in Fig. 9). It is, therefore, possible that these observations refer to the same gel → $L_{\beta I}$ phase transition detected for the DPPC bilayer as induced by ethanol. This transition is in fact predicted by MM simulations, as shown in Fig. 6 *B*. Above 80 mg/ml of ethanol, the T_m is observed to decrease again (Fig. 9). The reason for this is not completely clear. It is perhaps due, in part, to the dynamic replacement of the ternary complex of C(12):C(20)PC/1-hexanol/ethanol (1:1:1) by the binary mixture of C(12):C(20)PC/ethanol (1:2) at high ethanol concentrations. Our current understanding of the observed T_m change is rather limited; hence, no definitive explanation can be offered.

For aqueous dispersions of C(12):C(20)PC containing a fixed concentration of 1-octanol, the T_m profile is characterized by a sharp drop in T_m of 16.7°C at low ethanol concentrations (20 mg/ml) followed by a horizontal line connecting relatively constant T_m values (Fig. 9, *bottom curve*). These DSC results may be interpreted as being due to the 1-octanol/ethanol-induced gel → $L_{\beta I}$ phase transition at 20 mg/ml of ethanol, and the relatively stable $L_{\beta I}$ phase at higher ethanol concentrations. In the $L_{\beta I}$ gel phase, the ternary complex of C(12):C(20)PC/1-octanol/ethanol (1:1:1) is presumably formed. Furthermore, one may as-

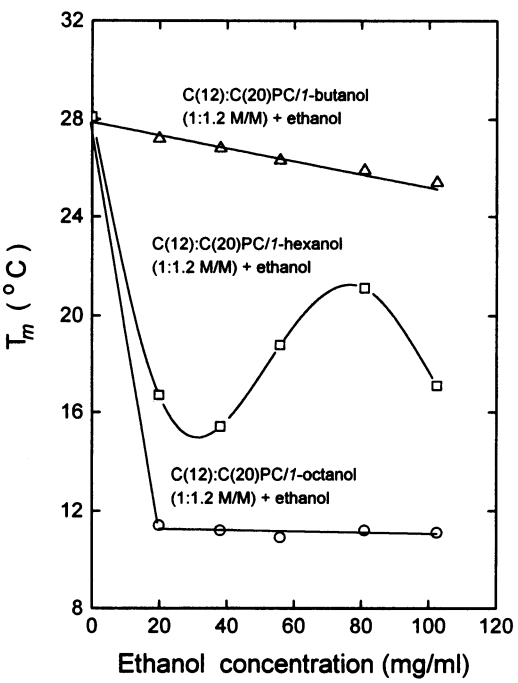


FIGURE 9 The plot of T_m versus ethanol concentration. The T_m values were obtained calorimetrically from aqueous dispersions of C(12):C(20)PC/*n*-alcohol (1:1.2 M/M) prepared in the presence of various concentrations of ethanol, where *n*-alcohol is 1-butanol, 1-hexanol, or 1-octanol.

sume that 1-octanol, because of its longer hydrocarbon chain, has a considerably higher stability in the hydrophobic pocket generated by the mismatched ΔC region between the two acyl chains in C(12):C(20)PC. Consequently, the ternary complex is stable over a wide concentration range of ethanol, and no significant exchange between 1-octanol and ethanol occurs in the ΔC region over the concentration range of ethanol studied. However, our understanding of the T_m profile is too premature to completely rule out other possible interpretations.

DISCUSSION

In recent years it has been shown quite exhaustively that lipid bilayer containing identical-chain phosphatidylcholines such as DPPC and DSPC can be induced by ethanol to undergo the partially interdigitated gel → fully interdigitated $L_{\beta I}$ gel phase transition at $T < T_m$ (Rowe, 1992). It is generally accepted that above a critical concentration, ethanols can perturb the lateral chain-chain interactions among PC molecules within the gel-state bilayer and, at the same time, ethanols can also displace H_2O molecules at the hydrocarbon/H_2O interface. These combined effects of ethanol can cause lipid chain interdigitation, resulting in the partially interdigitated gel → $L_{\beta I}$ phase transition. The molecular structure of the $L_{\beta I}$ phase has recently been identified by Adachi et al. (1995); this structural model is characterized by a basic building block composed of a binary mixture of DPPC/ethanol (1:2). An important feature of this building block is that one molecule of ethanol can fit into the hydrophobic pocket in the ΔC region, with the hydroxyl group of ethanol lying against the carbonyl oxygen of the neighboring lipid acyl chain, while the second molecule of ethanol is aligned in line with the longer acyl chain (Fig. 1 *D*). Inspection of the model suggests that ethanols can stabilize the fully interdigitated $L_{\beta I}$ bilayer by preventing the two acyl chains of DPPC molecule from directly contacting water. In the present work we have extended this molecular model to other mixed-chain phosphatidylcholines with different ΔC values. Specifically, we have carried out systematic MM calculations to simulate the structures and energies of various binary mixtures of C(*X*):C(*Y*)PC/ethanol (1:2) and ternary complexes of C(*X*):C(*Y*)PC/ethanol/*n*-alcohol (1:1:1). Despite the fact that many approximations have been made in our MM simulations, two important conclusions can be drawn from our computational results. First, when mixed-chain C(*X*):C(*Y*)PC are moderately symmetrical with ΔC values of <4.2 C-C bond lengths, these C(*X*):C(*Y*)PC can be induced by ethanol to undergo the partially interdigitated → $L_{\beta I}$ phase transition. In contrast, highly asymmetric PCs with $\Delta C > 4.2$ are preferably assembled into the partially interdigitated bilayer, which cannot be induced by ethanol to transform into the $L_{\beta I}$ phase. Second, for lipid bilayers composed of highly asymmetric PCs with $\Delta C = 7.0$, the partially interdigitated → $L_{\beta I}$ phase transition can be induced by 1-hexanol (or 1-octanol)/ethanol complex, because the longer chain *n*-alcohol is shown by MM calculations to fit much more snugly into the hydrophobic pocket in the ΔC region (Fig. 4 *D*).

It should be explicitly pointed out that our MM simulations have been performed based on crystal coordinates of DMPC. Many packing features of the lipid molecule detected in the gel-state bilayer such as the chain tilt are not considered. Hence, the conclusions drawn from MM simulations should be applied, strictly speaking, only to phospholipids packed in the crystalline state. Furthermore, the orientations of the lipid headgroup and acyl chains are different for PE and PC in the single crystals (Pascher et al., 1992); consequently, the simulation approach adopted in this paper should be used for crystalline phosphatidylcholines only. Nevertheless, we are interested primarily in the *relative* tendency of a homologous series of mixed-chain C(*X*):C(*Y*)PC constituting the bilayers in aqueous dispersions toward forming the $L_{\beta I}$ phase in the presence of high concentrations of alcohol. As such, we can take the computational results as our guide to design the relevant DSC experiments. Given the results of MM simulations, for instance, we have synthesized a homologous series of five molecular species of C(*X*):C(*Y*)PC with ΔC values within the range of 0.5 to 6.5 C-C bond lengths. DSC experiments are subsequently performed with the five molecular species of C(*X*):C(*Y*)PC, in excess H_2O, in the presence of various concentrations of ethanol. Results of these DSC experiments show clearly that only lipid dispersions prepared from C(*X*):C(*Y*)PC with gel-state ΔC values of <4.0 C-C bond lengths exhibit biphasic profiles in the plot of T_m versus ethanol concentration (Fig. 8). Furthermore, the lipid dispersions of C(12):C(20)PC ($\Delta C = 6.5$), which do not exhibit calorimetrically the ethanol-induced biphasic T_m profile, do show nonlinear behavior in the T_m versus ethanol concentration plot when a third component, 1-hexanol or 1-octanol, is present in the lipid dispersion (Fig. 9). Interestingly, all of these DSC results are indeed in excellent agreement with the expectations predicted by MM simulations. The work presented in this communication may thus serve as a paradigm to demonstrate that the molecular mechanics approach is a powerful method that can guide us to better understand the structures and properties of phospholipid assemblies. Finally, it should also be mentioned that none of the biphasic T_m curves observed in this study (Figs. 8 and 9) have yet been demonstrated to correlate directly with the partially interdigitated gel → $L_{\beta I}$ phase transition. In the future, experimental tests to demonstrate this correlation will be a rewarding endeavor.

We thank Mrs. Debbie Londeree Proffitt for typing the manuscript during the holiday season.

It is with deep gratitude and great admiration that we dedicate this paper to Professor Thomas E. Thompson on the occasion of his 70th birthday.

This work is supported, in part, by National Institutes of Health Grant GM17452.

REFERENCES

Adachi, T., H. Takahashi, K. Ohki, and I. Hatta. 1995. Interdigitated structure of phospholipid-alcohol systems studied by x-ray diffraction. *Biophys. J.* 68:1850–1855.

Allinger, N. L. 1977. Conformational anaylsis. 130. MM2. A hydrocarbon force field utilizing V_1 and V_2 torsional terms. *J. Am. Chem. Soc.* 99:8127–8134.

Huang, C., and S. Li. 1996. Computational molecular models of lipid bilayers containing mixed-chain saturated and monounsaturated acyl chains. *In* Handbook of Nonmedical Applications of Liposomes, Vol. I. D. D. Lasic and Y. Barenholz, editors. CRC Press, Boca Raton, FL. 173–194.

Huang, C., and J. T. Mason. 1986. Structure and properties of mixed-chain phospholipid assemblies. *Biochim. Biophys. Acta.* 864:423–470.

Li, S., and C. Huang. 1996. Molecular mechanics simulation studies of dienoic hydrocarbons: from alkenes to 1-palmitoyl-2-linoleoyl-phosphatidylcholines. *J. Comp. Chem.* 17:1012–1023.

Li, S., Z-q. Wang, H-n. Lin, and C. Huang. 1993. Energy-minimized structures and packing states of a homologous series of mixed-chain phosphatidylcholines: a molecular mechanics study on the diglyceride moieties. *Biophys. J.* 65:1415–1428.

Li, S., Z-q. Wang, H-n. Lin, and C. Huang. 1994. On the main phase transition temperatures of highly asymmetric mixed-chain phosphatidylcholines. *Biochim. Biophys. Acta.* 1194:271–280.

Lin, H-n., Z-q. Wang, and C. Huang. 1990. Differential scanning calorimetry study of mixed-chain phosphatidylcholines with a common molecular weight identical with diheptadecanoylphosphatidylcholines. *Biochemistry.* 29:7063–7072.

Löbbecke, L., and G. Cevc. 1995. Effects of short-chain alcohols on the phase behavior and interdigitation of phosphatidylcholine bilayer membranes. *Biochim. Biophys. Acta.* 1237:59–69.

Mason, J. T., A. V. Broccoli, and C. Huang. 1981. A method for the synthesis of isomerically pure saturated mixed-chain phosphatidylcholines. *Anal. Biochem.* 113:96–101.

Mena, P. L., and C. Djerassi. 1985. Synthesis of 5,9-hexacosadienoic acid phospholipids. 11. Phospholipid studies of marine organisms. *Chem. Phys. Lipids.* 37:257–270.

Ohki, K., K. Tamura, and I. Hatta. 1990. Ethanol induces interdigitated gel phase ($L_{\beta I}$) between lamellar gel phase ($L_{\beta'}$) and ripple phase ($P_{\beta'}$) in phosphatidylcholine membranes: a scanning density meter study. *Biochim. Biophys. Acta.* 1028:215–222.

Pascher, I., M. Lundmark, P-G. Nyholm, and S. Sundell. 1992. Crystal structures of membrane lipids. *Biochim. Biophys. Acta.* 1113:339–373.

Roth, L. G., and C-H. Chen. 1991. Thermodynamic elucidation of ethanol-induced interdigitation of hydrocarbon chains in phosphatidylcholine bilayer vesicles. *J. Phys. Chem.* 95:7955–7959.

Rowe, E. S. 1983. Lipid chain length and temperature dependence of ethanol-phosphatidylcholine interactions. *Biochemistry.* 22:3299–3305.

Rowe, E. S. 1992. Effects of alcohol on membrane lipids. *In* Alcohol: Neurobiology and Physiology. R. Watson, editor. CRC Press, Boca Raton, FL. 239–267.

Rowe, E. S., and J. M. Campion. 1994. Alcohol induction of interdigitation in distearoylphosphatidylcholine: fluorescence studies of alcohol chain length requirements. *Biophys. J.* 67:1888–1895.

Simon, S. A., and T. J. McIntosh. 1984. Interdigitated hydrocarbon chain packing causes the biphasic transition behavior in lipid/alcohol dispersions. *Biochim. Biophys. Acta.* 773:169–172.

Wang, Z-q., H-n. Lin, S. Li, and C. Huang. 1995. Phase transition behavior and molecular structures of monounsaturated phosphatidylcholines. *J. Biol. Chem.* 270:2014–2023.