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Interconversion of bilayer phase transition temperatures between phosphatidylethanolamines and phosphatidylcholines

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High-resolution differential scanning calorimetric studies were performed to investigate the thermotropic phase behavior of 31 molecular species of phosphatidylethanolamines in excess water. Upon reheating, the aqueous dispersions of these lipids undergo the gel to liquid-crystalline phase transitions at well defined temperatures (T_m). These T_m values were shown to relate to the structural parameters of the underlying lipid molecules in a characteristic manner. Based on these observations, an interconversion of the T_m values between saturated phosphatidylethanolamines and phosphatidylcholines is established quantitatively for the first time.

Introduction

The lipid bilayer of saturated phosphatidylcholines in excess water undergoes a highly cooperative gel to liquid-crystalline phase transition at a characteristic temperature, T_m, upon heating. Recently, a multiple regression analysis of the T_m values for 44 molecular species of saturated phosphatidylcholines in terms of two structural parameters of these lipid molecules has led to the establishment of a general relationship between the T_m and the structural parameters for all relevant phosphatidylcholines [1]. Interestingly, such a general structure-property relationship has also been derived theoretically based on the thermodynamic analysis of lipid phase transitions [2]. Bilayers composed of saturated phosphatidylethanolamines also undergo the sharp gel to liquid-crystalline phase transitions. The question of whether the T_m values of phosphatidylethanolamine bilayers can be related to the structural parameters of the underlying lipid molecules was investigated in this study. Here, we have demonstrated that the T_m values obtained calorimetrically from 31 different phosphatidylethanolamine bilayers can indeed be related systematically to the structural parameters of the corresponding lipid molecules. Furthermore, an interconversion of the T_m values between saturated phosphatidylethanolamines and phosphatidylcholines is established quantitatively for the first time. These calorimetric results are of biological interest because both phosphatidylcholines and phosphatidylethanolamines are major lipid components of cell membranes; consequently, the structure-property relationships established for these lipids may be considered as prototype models for elucidating the various phases and their transitions found in cell membranes.

Materials and Methods

Materials. Twenty-two molecular species of saturated mixed-chain phosphatidylethanolamines, or C(X):C(Y)PE (saturated phosphatidylethanolamines with X and Y carbon atoms in the *sn*-1 and *sn*-2 acyl chains, respectively), were semi-synthesized, via the phospholipase D-catalyzed transphosphatidylation, from the corresponding mixed-chain phosphatidylcholines in the presence of ethanolamine, and the synthesized lipids were purified by column chromatography on silica gel [3,4]. Mixed-chain phosphatidylcholines were semi-synthesized by the 4-pyrrolidinopyridine-catalyzed acylation of the appropriate lysophosphatidylcholines and fatty acid anhydrides according to the method used previously [5].

High-resolution differential scanning calorimetry (DSC). The phase transitions of aqueous lipid dispersions were studied using a high-resolution MC2 differential scanning calorimeter (Microcal, Northampton, MA) as described eleswhere [5]. The lipid samples used for DSC studied were prepared according to

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Koynova and Hinz [6]. Prior to the initial DSC run, an incubation time of 90 min was allowed for the degassed sample in the sample cell.

The structural parameters. The two structural parameters chosen in this study were (1) the effective chain-length difference between the two acyl chains (ΔC) for lipids in the gel-state bilayer, and (2) the thickness of the hydrocarbon core of the gel-state lipid bilayer (N). For mixed-chain phospholipids such as C(X):C(Y)PE, ΔC and N are related to X and Y as follows: $\Delta C = |X - Y + 1.5|$ and $N = X + Y - 0.5$ [1,7]. The units for both parameters are C-C bond lengths.

Results and Discussion

The phase transition characteristics observed calorimetrically for aqueous dispersions prepared individually from a homologous series of seven identical-chain phosphatidylethanolamines ranging from C(11):C(11) PE to C(17):C(17)PE and other 22 mixed-chain C(X):C(Y)PE are basically similar. In the initial DSC heating scans, all samples exhibit a symmetric sharp peak; however, the same samples show a smaller and down-shifted peak upon immediate second and subsequent heating scans. These typical phase characteristics, represented by C(12):C(12)PE dispersions, are shown in Fig. 1. The transition observed in the initial DSC heating scan is assigned to the crystalline to liquid-crystalline phase transition, and the transition observed in the second or subsequent DSC heating scans is the gel to liquid-crystalline phase transition [8–10]. For longer chain phosphatidylethanolamines such as C(18):C(18)PE and C(20):C(20)PE, an additional broad and smaller transition is also detected in the initial DSC heating scans (Fig. 1). The low- and high-temperature transitions observed in the initial DSC scans for C(18):C(18)PE or C(20):C(20)PE dispersions correspond to the crystalline to tilted gel phase and the tilted gel to liquid-crystalline phase transitions, respectively [11]. Because of the irreversible nature of the crystalline to gel phase transition, we have studied only the phase characteristics of the reversible phase transitions observed in the second or subsequent DSC heating scans. In particular, we have chosen to report in this communication the dependence of the phase transition temperatures, T_m, on the two structural parameters (ΔC and N).

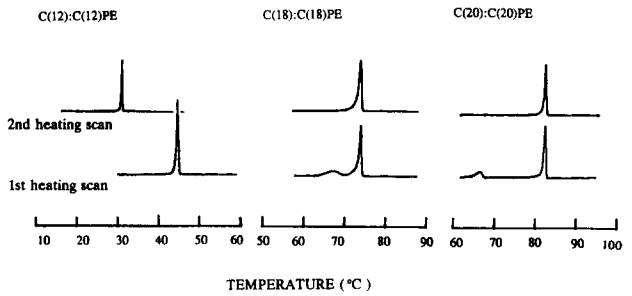


Fig. 1. The initial and second DSC heating scans of C(12):C(12)PE (A), C(18):C(18)PE (B), and C(20):C(20)PE (C). The DSC experiments were carried out with a high-resolution MC-2 differential scanning calorimeter as described previously [3]. All buffered aqueous solutions used for preparing lipid samples (3–6 mM) contained 5 mM phosphate buffer (pH 7.4), 1 mM EDTA, and 50 mM NaCl.

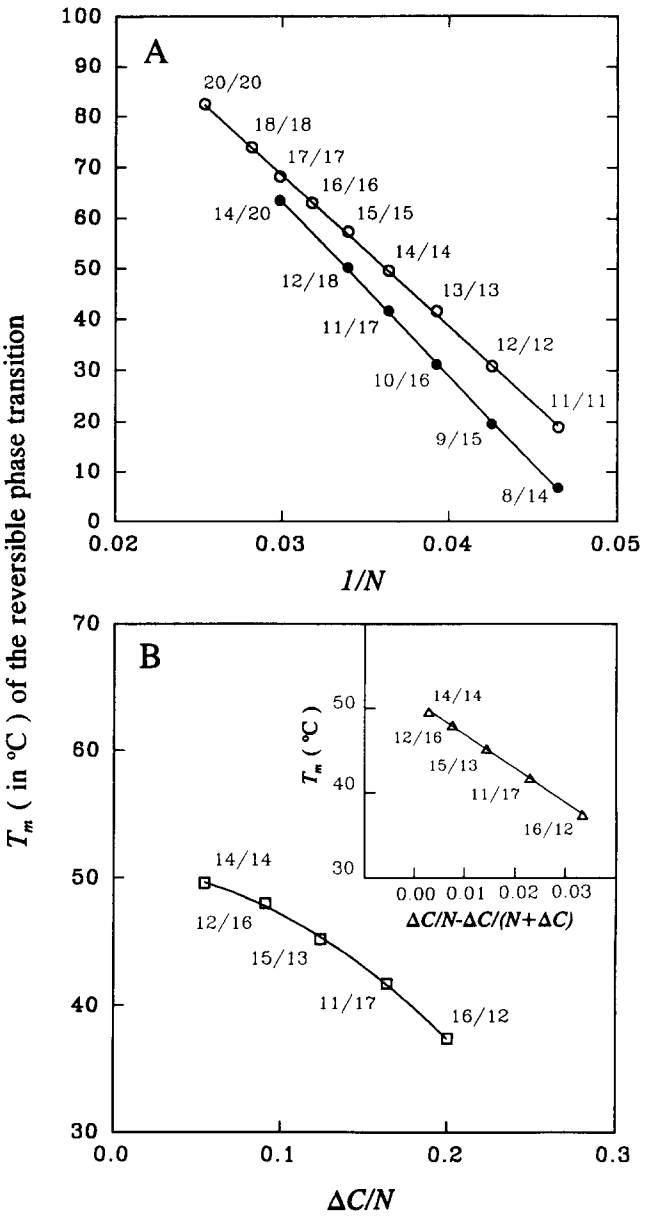


Fig. 2. Plots of the phase transition temperature versus the structural parameters. (A) The T_m values from two series of phosphatidylethanolamines with $\Delta C = 1.5$ and 4.5 are plotted against $1/N$. (B) The T_m values of mixed-chain phosphatidylethanolamines with the same N values are plotted against $\Delta C/N$. The same data are plotted against $[\Delta C/N - \Delta C/(N + \Delta C)]$ in the inset. Each lipid is represented by X/Y, where X and Y are the total numbers of carbons in the *sn*-1 and *sn*-2 acyl chains, respectively.

The T_m values of a series of nine identical-chain C(X):C(X)PE with a common ΔC value of 1.5 and a series of 6 mixed-chain C(X):C($X+6$)PE with a common ΔC value of 4.5 are plotted in Fig. 2A against $1/N$. A least-squares line with a correlation coefficient of 0.9998 is obtained for each of the two data sets. In Fig. 2B, the T_m values of a series of five phosphatidylethanolamines with a common value of $N = 27.5$ are plotted against $\Delta C/N$, and a smooth curvilinear line is obtained. If, however, a correction term of $\Delta C/(N+\Delta C)$ is subtracted from the value of $\Delta C/N$, then the same T_m values can be connected by a least-squares line with a correlation coefficient of 0.9996 as shown in the inset in Fig. 2B. Based on the results shown in Fig. 2, one can draw the following two conclusions: (1) For members of a homologous series of phosphatidylethanolamines with a common ΔC value, the larger the $1/N$ value, the smaller is the T_m value (Fig. 2A); moreover, the $1/N$-dependence of T_m is a linear one. (2) For lipids with the same common N value, the larger the ΔC value, the smaller is the T_m value (Fig. 2B); furthermore, the T_m value is a linear function of $[\Delta C/N - \Delta C/(N+\Delta C)]$. These relationships can be approximated by the following equation:

$$T_m = a_o - a_1(1/N) - a_2(\Delta C/N) + a_2\Delta C/(N+\Delta C) \quad (1)$$

Here, the first term a_o is the extrapolated T_m value for lipids with an infinite bilayer thickness ($N \to \infty$). The second term corresponds to the contribution of the thickness of the hydrocarbon core of the bilayer and this can be considered as the dominant term in determining the chain melting temperature; hence, the coefficient a_1 must be considerably greater than a_2 [1]. The third term is the perturbation term contributed by the lipid asymmetry (ΔC) which is modulated by the last term, a correction term. For a homologous series of lipids with a constant ΔC value, Eqn. 1 can be reduced to $T_m = a_o - a_1'(1/N)$, since the last term in Eqn. 1 can be dropped out due to $a_1 \gg a_2$. The two linear curves with negative slopes shown in Fig. 2A are thus consistent with the results described by Eqn. 1. When N is constant, Eqn. 1 reduces to $T_m = a_o' - a_2[\Delta C/N - \Delta C/(N+\Delta C)]$, and the data shown in the inset of Fig. 2B are in complete accord with the results formulated by Eqn. 1. Once we have established the approximate relationship between the T_m and the structural parameters as given by Eqn. 1, all experimental T_m values can now be subjected to the multiple regression analysis in an attempt to establish a quantitative structure-property relationship for most C(X):C(Y)PE.

In a previous study of mixed-chain phosphatidylcholines or C(X):C(Y)PC, it was noted that the phase transition behavior of C(X):C(Y)PC with $X \geq Y$ is slightly different from that of C(X):C(Y)PC with $X < Y$ [1]. Here, we also divide the mixed-chain phosphatidylethanolamines into two groups accordingly. Of the 31 T_m values determined in this study, 19 are obtained with C(X):C(Y)PE with a longer *sn*-1 acyl chain, and the other 12 are from those with a longer *sn*-2 acyl chain. Thirty of these T_m values are presented in Fig. 3A and the remaining one is 6.7°C for C(8):C(14)PE. Based on these T_m values together with the corresponding ΔC and N values and the approximate form of Eqn. 1, two general equations can be obtained from the multiple regression approach as follows:

For phosphatidylethanolamines with a longer effective *sn*-1 acyl chain,

$$T_m = 159.85 - 2993.84(1/N) - 330.75(\Delta C/N) + 317.65\Delta C/(N+\Delta C) \quad (2)$$

with $\sigma = 0.9992$ and RMSE = 0.6923, and, for lipids with a longer effective *sn*-2 acyl chain,

$$T_m = 159.55 - 2957.10(1/N) - 298.04(\Delta C/N) + 271.60\Delta C/(N+\Delta C) \quad (3)$$

with $\sigma = 0.9997$ and RMSE = 0.4478, where σ is the correlation coefficient and RMSE is the root mean square error of the statistical analysis.

A total of 213 predicted T_m values calculated for various phosphatidylethanolamines based on Eqns. 2 and 3 is presented in Fig. 3A. These equations are applied only to those C(X):C(Y)PE which are known to undergo the partially interdigitated gel to liquid-crystalline phase transitions; hence, there are 111 blank spaces in Fig. 3A. A comparison between the experimental and calculated T_m values, shown in Fig. 3A, indicates that, of the 31 data pairs, only two lipids display deviations of greater than 1 C°, and the largest deviation of 1.2 C° corresponds to a relative error of 0.4% in absolute temperature for C(13):C(11)PE. It should be emphasized that the predicted T_m value for a C(X):C(Y)PE bilayer is calculated, using Eqn. 2 or 3, from the values of N and ΔC, which, in turn, are calculated from the values of X and Y using the following relations [1,7]: $\Delta C = |X - Y + 1.5|$ and $N = X + Y - 0.5$. Ultimately, the T_m value of a lipid bilayer of C(X):C(Y)PE is related to the total numbers of carbons in the *sn*-1 and *sn*-2 acyl chains (X and Y) of C(X):C(Y)PE. This dependence of T_m on X and Y is illustrated in Fig. 3A.

Since the publication of 44 T_m values for C(X):C(Y)PC [1], six additional T_m values have been determined calorimetrically in this laboratory. These 50 T_m values are presented in Fig. 3B. Based on these values, two general equations are derived for phosphatidylcholines as follows:

A

Total Carbons in the *sn*-1 Acyl Chain of C(X):C(Y)PE	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
26												*84.5*	*88.2*	*91.5*	*94.4*	*97.0*	*99.2*	*101.1*
25											*80.7*	*84.7*	*88.3*	*91.5*	*94.2*	*96.6*	*98.7*	*100.4*
24										*76.6*	*81.0*	*84.9*	*88.3*	*91.3*	*93.9*	*96.1*	*98.0*	*99.5*
23									*71.9*	*76.8*	*81.0*	*84.8*	*88.1*	*90.9*	*93.3*	*95.3*	*97.0*	*97.5*
22								*68.8* **67.9**	*72.1*	*76.8*	*80.9*	*84.5*	*87.6*	*90.2*	*92.4*	*94.2*	*94.8*	*95.2*
21							*61.0*	*66.9*	*72.1*	*76.7*	*80.6*	*84.0*	*86.8*	*89.2*	*91.2*	*91.9*	*92.3*	*92.5*
20						*54.5*	*61.1*	*66.9* **67.6**	*71.9*	*76.3*	*80.0*	*83.1* **82.5**	*85.7*	*87.9*	*88.7*	*89.2*	*89.5*	*89.5*
19						*54.5*	*61.0*	*66.6*	*71.4*	*75.5*	*79.0*	*81.9*	*84.3*	*85.2*	*85.8*	*86.1*	*86.2*	*86.1*
18					*47.0*	*54.3* **54.9**	*60.6*	*66.0*	*70.6*	*74.4* **74.0**	*77.6*	*80.3*	*81.3*	*82.0*	*82.4*	*82.5*	*82.5*	*82.3*
17					*46.6*	*53.7*	*59.8*	*65.0*	*69.3* **68.2**	*72.8*	*75.8*	*77.0*	*77.8*	*78.3*	*78.5*	*78.5*	*78.4*	*78.1*
16				*37.8* **37.4**	*45.9*	*52.8* **52.3**	*58.6*	*63.5* **63.0**	*67.5*	*70.8*	*72.2*	*73.1*	*73.7*	*74.0*	*74.1*	*74.0*	*73.8*	*73.4*
15			*27.5* **26.8**	*36.8*	*44.7* **45.2**	*51.4*	*56.3* **57.3**	*61.4*	*65.2*	*66.8*	*67.9*	*68.6* **68.0**	*69.0*	*69.2*	*69.2*	*69.0*	*68.7*	
14		*15.3*	*26.2*	*35.4* **34.6**	*43.1*	*49.4* **49.6**	*54.5* **55.0**	*58.8*	*60.7*	*62.0* **61.6**	*62.9*	*63.4* **63.5**	*63.7*	*63.7*	*63.6*	*63.3* **63.7**		
13		*13.6*	*24.4* **23.2**	*33.3*	*40.6* **41.5**	*46.5*	*51.5*	*53.7*	*55.3*	*56.4* **56.2**	*57.1*	*57.4*	*57.6*	*57.5*				
12		*11.2*	*21.8*	*31.4* **30.8**	*37.3*	*43.0*	*45.6*	*47.5* **48.0**	*48.9*	*49.8* **50.3**	*50.3*	*50.6*						
11	*-4.7*	*8.0*	*18.2* **18.9**	*26.3*	*33.0*	*36.3*	*38.6*	*40.3*	*41.4* **41.7**	*42.2*	*42.7*							
10	*-8.9*	*3.6*	*13.3*	*21.3*	*25.2*	*28.1* **28.8**	*30.2*	*31.7* **31.2**	*32.7*									
9	*-14.5*	*-2.6*	*7.1*	*12.0*	*15.6*	*18.3*	*20.3* **19.6**											

Total Carbons in the *sn*-2 Acyl Chain of C(X):C(Y)PE

Fig. 3. Calculated and experimental T_m values for bilayers of phosphatidylethanolamines (A) and phosphatidylcholines (B). Each rectangular box represents a molecular species of C(X):C(Y)PE (A) or C(X):C(Y)PC (B), where the values of X and Y are specified on the vertical and horizontal axes, respectively. The experimental and calculated T_m values are given in bold-face and italic, respectively.

For phosphatidylcholines with a longer effective *sn*-1 acyl chain,

$$T_m = 161.97 - 3716.03(1/N) - 292.03(\Delta C/N) + 256.46\Delta C/(N + \Delta C) \quad (4)$$

with $\sigma = 0.9997$ and RMSE = 0.3923, and for C(X):C(Y)PC with a longer effective *sn*-2 acyl chain,

$$T_m = 155.47 - 3537.16(1/N) - 218.50(\Delta C/N) + 165.44\Delta C/(N + \Delta C) \quad (5)$$

with $\sigma = 0.9994$ and RMSE = 0.5448.

A total of 213 predicted T_m values for C(X):C(Y)PC is given in Fig. 3B. The difference in the predicted T_m values between C(X):C(Y)PE and C(X):C(Y)PC, $\Delta T_m^{PE-PC} = T_m^{PE} - T_m^{PC}$, for all 213 pairs of data shown in Fig. 3A and B can be further correlated with their respective values of ΔC and N, leading to the following two equations:

For lipids with a longer effective *sn*-1 acyl chain,

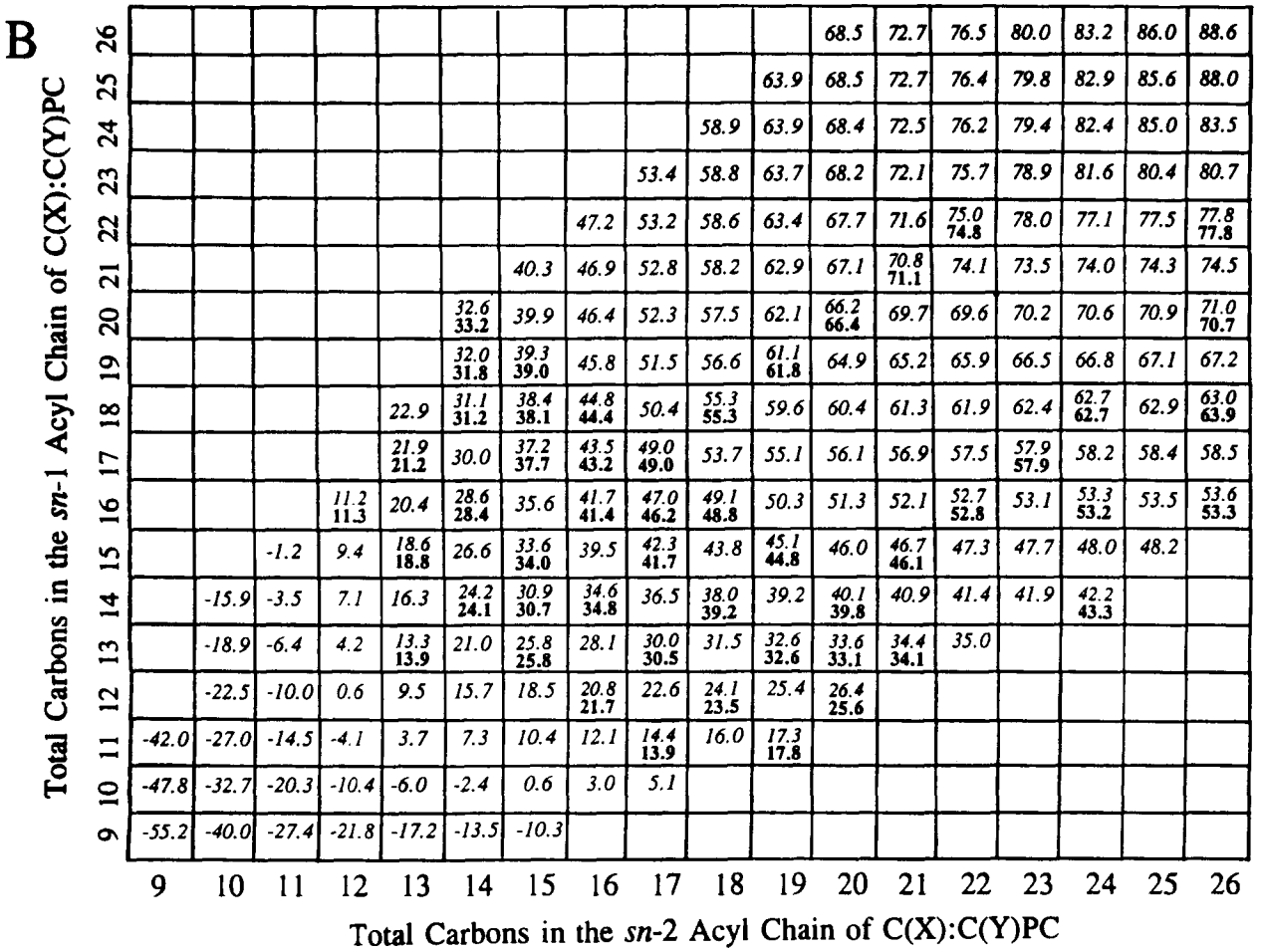
$$\Delta T_m^{PE-PC} = -2.12 + 721.92(1/N) - 38.93(\Delta C/N) + 61.53\Delta C/(N + \Delta C) \quad (6)$$

with $\sigma = 1.0000$ and RMSE = 0.0306, and, for lipids with a longer effective *sn*-2 acyl chain,

$$\Delta T_m^{PE-PC} = 4.09 + 579.94(1/N) - 79.75(\Delta C/N) + 106.39\Delta C/(N + \Delta C) \quad (7)$$

with $\sigma = 1.0000$ and RMSE = 0.0265.

Eqns. 6 and 7 yield the ΔT_m^{PE-PC} values which can then be employed to estimate the T_m values for phosphatidylcholines based on experimentally determined T_m values of phosphatidylethanolamines or vice versa. In this study, we have determined 31 T_m values for phosphatidylethanolamines. In addition, a total of 50 experimentally determined T_m values for phosphatidylcholines is also presented. Between these two sets of data, 18 pairs of T_m values for phosphatidylethanolamines and phosphatidylcholines with the same chemical structure of acyl chains are identified (Table I). The calculated T_m^{PC} values for phosphatidylcholines based on the ΔT_m^{PE-PC} values derived from Eqns. 6 and 7 and the experimental T_m^{PE} values of phosphatidylethanolamines are also summarized in Table I. Clearly,



	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
26												68.5	72.7	76.5	80.0	83.2	86.0	88.6
25											63.9	68.5	72.7	76.4	79.8	82.9	85.6	88.0
24										58.9	63.9	68.4	72.5	76.2	79.4	82.4	85.0	83.5
23									53.4	58.8	63.7	68.2	72.1	75.7	78.9	81.6	80.4	80.7
22								47.2	53.2	58.6	63.4	67.7	71.6	75.0 74.8	78.0	77.1	77.5	77.8 77.8
21							40.3	46.9	52.8	58.2	62.9	67.1	70.8 71.1	74.1	73.5	74.0	74.3	74.5
20						32.6 33.2	39.9	46.4	52.3	57.5	62.1	66.2 66.4	69.7	69.6	70.2	70.6	70.9	71.0 70.7
19						32.0 31.8	39.3 39.0	45.8	51.5	56.6	61.1 61.8	64.9	65.2	65.9	66.5	66.8	67.1	67.2
18					22.9	31.1 31.2	38.4 38.1	44.8 44.4	50.4	55.3 55.3	59.6	60.4	61.3	61.9	62.4	62.7 62.7	62.9	63.0 63.9
17					21.9 21.2	30.0	37.2 37.7	43.5 43.2	49.0 49.0	53.7	55.1	56.1	56.9	57.5	57.9 57.9	58.2	58.4	58.5
16				11.2 11.3	20.4	28.6 28.4	35.6	41.7 41.4	47.0 46.2	49.1 48.8	50.3	51.3	52.1	52.7 52.8	53.1	53.3 53.2	53.5	53.6 53.3
15			-1.2	9.4	18.6 18.8	26.6	33.6 34.0	39.5	42.3 41.7	43.8	45.1 44.8	46.0	46.7 46.1	47.3	47.7	48.0	48.2	
14		-15.9	-3.5	7.1	16.3	24.2 24.1	30.9 30.7	34.6 34.8	36.5	38.0 39.2	39.2	40.1 39.8	40.9	41.4	41.9	42.2 43.3		
13		-18.9	-6.4	4.2	13.3 13.9	21.0	25.8 25.8	28.1	30.0 30.5	31.5	32.6 32.6	33.6 33.1	34.4 34.1	35.0				
12		-22.5	-10.0	0.6	9.5	15.7	18.5	20.8 21.7	22.6	24.1 23.5	25.4	26.4 25.6						
11	-42.0	-27.0	-14.5	-4.1	3.7	7.3	10.4	12.1	14.4 13.9	16.0	17.3 17.8							
10	-47.8	-32.7	-20.3	-10.4	-6.0	-2.4	0.6	3.0	5.1									
9	-55.2	-40.0	-27.4	-21.8	-17.2	-13.5	-10.3											

Fig. 3 (continued).

TABLE I

Interconversion of T_m between phosphatidylethanolamines and phosphatidylcholines

The various values shown in the third column are the calculated ΔT_m^{PE-PC} values based on Eqns. 6 and 7 in the text. The calculated T_m^{PC} values, shown in the fourth column, are obtained by subtracting the values given in the second column from the corresponding values given in the third column. The last column shows the difference in T_m between the calculated and experimental values in either C° or in relative % error, also in C°.

Lipid chain structure	Experimental T_m^{PE} (°C)	ΔT_m^{PE-PC} (C°)	Calculated T_m^{PC} (°C)	Experimental T_m^{PC} (°C)	ΔT_m^{PC} in C° (or %C°)
C(13):C(13)	41.5	27.3	14.2	13.9	0.3 (2.1%)
C(14):C(14)	49.6	25.2	24.4	24.1	0.3 (1.2%)
C(15):C(15)	57.3	23.4	33.9	34.0	−0.1 (−0.3%)
C(16):C(16)	63.0	21.7	41.3	41.4	−0.1 (−0.2%)
C(17):C(17)	68.2	20.3	47.9	49.0	−1.1 (−2.3%)
C(18):C(18)	74.0	19.1	54.9	55.3	−0.4 (−0.7%)
C(20):C(20)	82.5	16.9	65.6	66.4	−0.8 (−1.2%)
C(16):C(12)	37.4	26.6	10.8	11.3	−0.5 (−4.6%)
C(11):C(17)	41.7	27.1	14.6	13.9	0.7 (4.8%)
C(15):C(13)	45.2	26.5	19.1	18.8	0.3 (1.6%)
C(12):C(16)	48.0	26.8	21.2	21.7	−0.5 (−2.4%)
C(12):C(18)	50.3	25.7	24.6	23.5	1.1 (4.5%)
C(16):C(14)	52.3	24.3	28.0	28.4	−0.4 (−1.4%)
C(18):C(14)	54.9	23.1	31.8	31.2	0.6 (1.9%)
C(14):C(15)	55.0	23.6	31.4	30.7	0.7 (2.2%)
C(14):C(18)	61.6	24.0	37.6	39.2	−1.6 (−4.3%)
C(14):C(20)	63.5	23.3	40.2	39.8	0.4 (1.0%)
C(14):C(24)	63.7	21.1	42.6	43.3	−0.7 (−1.6%)

the agreements between the calculated and experimental values shown in column 6, Table I are excellent. The ability to predict T_m values for phosphatidylcholines from phosphatidylethanolamines or vice versa could be extremely useful. For instance, the T_m value of C(11):C(11)PC will be difficult to obtain calorimetrically because of the freeze of the sample. Based on the experimental T_m value of C(11):C(11)PE (18.9°C) and the calculated value of ΔT_m^{PE-PC} (32.8 C°), the T_m value for C(11):C(11)PC can be estimated to be −13.9°C.

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