Empirical Estimation of the Gel to Liquid-Crystalline Phase Transition Temperatures for Fully Hydrated Saturated Phosphatidylcholines[†]

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ABSTRACT: Phospholipids are a major component of biological membranes. In excess water, phospholipids may self-assemble into fully hydrated lamellae which, upon heating, may undergo the gel to liquid-crystalline phase transition at the characteristic temperature, T_m. Our present knowledge about the T_m values for various phospholipids is far from complete, although it is necessary to know the T_m value for preparing liposomes. In this study, we have derived empirically a general expression of $T_m = 154.2 + 2.0(\Delta C) - 142.8(\Delta C/CL) - 1512.5(1/CL)$ in which two apparent structural parameters, ΔC and CL, of a phosphatidylcholine molecule and their ratio, $\Delta C/CL$, are applied to estimate the T_m value of the phosphatidylcholine bilayer in excess water. The parameter ΔC is the effective chain-length difference, in C–C bond lengths, between the two acyl chains for the phosphatidylcholine molecule in the gel-state bilayer, and CL is the effective length of the longer of the two acyl chains, also in C–C bond lengths. A figure containing 163 calculated T_m values is presented, and this information will be useful as a guide for designing experiments.

When fully hydrated diacylphospholipids are subjected to heating, these lipids may undergo multiple thermotropic phase transitions at well-defined discrete temperatures (Chapman et al., 1967; Mabrey & Sturtevant, 1976; Chen et al., 1980; Wong, 1986). Of the several transitions, the main phase transition or the gel → liquid-crystalline phase transition is perhaps most interesting; this phase transition involves not only a marked positive change in entropy, enthalpy, and partial molar volume of the lipid bilayer in excess water (Nagle & Wilkinson, 1978) but also the onset of a number of highly cooperative intra- and intermolecular motions of phospholipids in the two-dimensional plane of the bilayer (Seelig & Seelig, 1980; Thompson & Huang, 1986; Pfeiffer et al., 1989). Moreover, the headgroup hydration is also increased at the main phase transition temperature or T_m (Ruocco & Shipley, 1982). In fact, enclosed multilamellar liposomes are well-known to form only after the phospholipids in excess water are heated above the T_m (Bangham et al., 1965; Singer et al., 1990). It is now a common practice that aqueous samples of phospholipids are prepared by dispersing first the lyophilized lipid in a buffered solution, followed by several cycles of heating/cooling of the dispersion at temperatures above and below the T_m. Using this procedure, one can be sure that the phospholipid under study is fully hydrated and that enclosed multilamellar liposomes are generated. It should be pointed out that it is this fully hydrated phospholipid bilayer with highly dynamic nature that has been widely employed as a model membrane system in studying the fundamental roles of membrane lipids in biological processes (Yeagle, 1987). Consequently, if one attempts to prepare model membranes from phospholipids, it is necessary to know, at least approximately, the T_m value of the phospholipid dispersion as a guide for designing experiments.

There are a huge variety of molecular species of phospholipids. Even if we consider only the saturated diacylphosphatidylcholines with the chain length in each acyl chain limited to a range of 9–26 carbons, there are still totally 324 possible species. The values of T_m of about 10 identical-chain phosphatidylcholines have been determined by high-resolution differential scanning calorimetry or DSC (Silvius, 1982; Small, 1986; Lewis et al., 1987). The thermotropic properties of less than 35 mixed-chain phosphatidylcholines have been elucidated by high-resolution DSC (Huang & Mason, 1986; Mattai et al., 1987; Xu & Huang, 1987; Shah et al., 1990; Lin et al., 1990; Wang et al., 1990). Compared to the total number of molecular species of saturated diacylphosphatidylcholines, it is obvious that our present knowledge about the T_m values of various saturated diacylphosphatidylcholines is far from complete. Recently, the T_m values of a number of identical molecular weight (MW) phosphatidylcholines and of two homologous series of phosphatidylcholines with fixed chain-length differences between their acyl chains have been fit successfully to the linear function of normalized chain-length difference ($\Delta C/CL$), when the values of $\Delta C/CL$ are within the range of 0.09–0.40 (Huang, 1990; Lin et al., 1990; Wang et al., 1990). It appears likely that an empirical estimation of a large number of unknown values of T_m may be possible by using these linear relationships and the limited T_m values of known phosphatidylcholines. In this paper, empirical linear relationships between the T_m and $\Delta C/CL$ and between the T_m and $1/CL$ are further analyzed, and these linear relationships are found to be the special cases of a more general expression whereby the values of T_m of a wide variety of fully hydrated diacylphosphatidylcholines may be estimated, provided that the values of the normalized chain-length difference ($\Delta C/CL$) between the *sn*-1 and *sn*-2 acyl chains of these phosphatidylcholines are within the range of 0.09–0.40. Here, the term ΔC is the effective chain-length difference, in C–C bonds, between the two acyl chains, and the term CL is the effective length of the longer of the two acyl chains, also in C–C bonds (Mason et al., 1981; Huang, 1990).

STRUCTURAL PARAMETERS AND DATA ANALYSES

The two acyl chains of phosphatidylcholine molecules in various aggregated states are well-known to exhibit different conformations (Yeagle, 1987). Specifically, the initial segment

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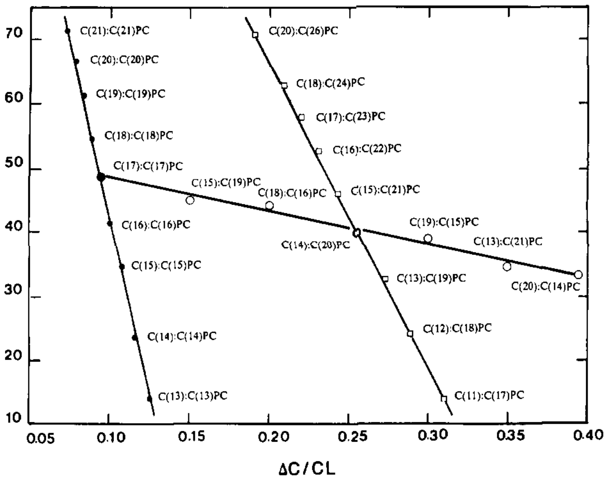

FIGURE 1: Linear relationship between the T_m values and the values of normalized chain-length difference ($\Delta C/CL$) for various phosphatidylcholines. The two vertically slanted lines are the least-squares lines connecting the T_m values for C(X):C(X)PC (solid circles) and C(X):C(X+6)PC (open squares) as reported by Lewis et al. (1987) and by Wang et al. (1990), respectively. The nearly horizontal line is the least-squares line fitting the T_m values for seven molecular species of saturated phosphatidylcholines whose molecular weights are identical (762.2). The T_m values for these identical MW phosphatidylcholines are taken from Lin et al. (1990), and the least-squares expression is $T_m = 53.9 - 52.8(\Delta C/CL)$.

of the sn-2 acyl chain runs parallel to the bilayer surface and then bends over at the C-2 position. Because of the bend in the sn-2 acyl chain, the two acyl chains run nearly parallel in the bilayer. In addition, the two acyl chains are different in their apparent chain lengths, being separated along the long molecular axis by 1.5 carbon–carbon bond lengths for identical-chain phosphatidylcholine in the gel-state bilayer (Zaccai et al., 1979). The normalized chain-length difference between the sn-1 and sn-2 acyl chains for a phosphatidylcholine molecule in the gel-state bilayer may be quantitatively expressed as $\Delta C/CL$ (Mason et al., 1981; Huang, 1990). In calculating the values of ΔC and CL, an inherent shortening of 1.5 C–C bond lengths for the sn-2 acyl chain must be taken into account. In the case of C(16):C(16)PC, for instance, the value of ΔC is 1.5 C–C bond lengths, and the value of CL is 15, corresponding to the length of the longer sn-1 acyl chain in C–C bonds. The normalized chain-length difference, $\Delta C/CL$, is thus 0.100. By taking C(13):C(19)PC as a second example, the values of ΔC, CL, and $\Delta C/CL$ are 4.5, 16.5, and 0.273, respectively.

Recently, the T_m values of aqueous dispersions prepared from C(17):C(17)PC, C(15):C(19)PC, C(18):C(16)PC, C(14):C(20)PC, C(19):C(15)PC, C(13):C(21)PC, and C(20):C(14)PC have been shown to be linearly related to the corresponding values of $\Delta C/CL$ for these identical MW phospholipids, and the values of $\Delta C/CL$ for these various lipids are in the range of 0.09–0.40 (Huang, 1990; Lin et al., 1990). In addition, the T_m values reported by Lewis et al. (1987) and by Wang et al. (1990) for a homologous series of identical-chain phosphatidylcholines, C(X):C(X)PC, and for a similar series of mixed-chain phosphatidylcholines, C(X):C(X+6)PC, have also been shown to be linearly related to the function of $\Delta C/CL$. These linear relationships are shown graphically in Figure 1.

By fitting the respective experimental data of Lewis et al. (1987) and of Wang et al. (1990) as a function of $\Delta C/CL$, two least-squares expressions of $T_m = 156.9 - 1148.3(\Delta C/CL)$ and $T_m = 163.3 - 481.1(\Delta C/CL)$ are obtained, respectively, and each best-fit straight line has a correlation coefficient of 0.999. The standard deviations of the slope and the intercept for the first linear least-squares line fitting the data of Lewis et al. (1987) are 12.2 and 1.2, respectively, and those for the second linear least-squares line are 4.7 and 1.2, respectively. The ΔC values for C(X):C(X)PC and C(X):C(X+6)PC are constant; they are 1.5 and 4.5, respectively. The best-fit linear expressions can thus be reduced to the forms:

$$\text{C}(X)\text{:C}(X+6)\text{PC:}\quad T_m = (163.4 \pm 0.8) - (2165.7 \pm 14.0)(1/CL)$$

$$\text{C}(X)\text{:C}(X)\text{PC:}\quad T_m = (157.2 \pm 0.9) - (1725.9 \pm 14.7)(1/CL)$$

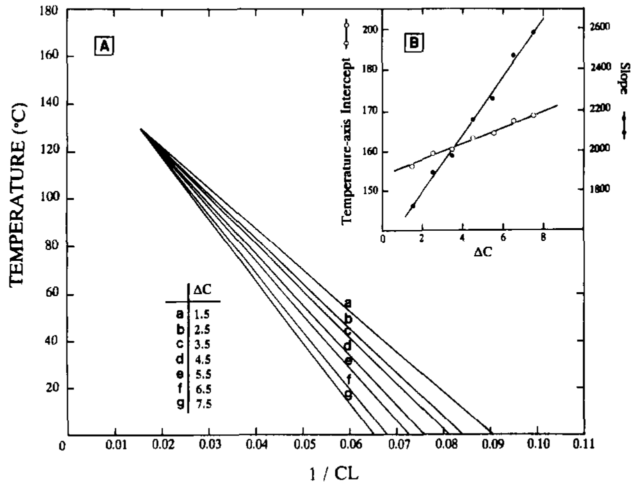

FIGURE 2: (A) T_m values for saturated diacylphosphatidylcholines with fixed values of ΔC versus the inverse of CL. Lines a and d are the least-squares lines fitting the T_m values for C(X):C(X)PC (ΔC = 1.5) and C(X):C(X+6)PC (ΔC = 4.5), respectively. The point of intersection of these two least-squares lines defines a unique pair of T_m and $1/CL$ values, and this point is then used as a fixed point to generate a family of lines by connecting it to the various pairs of T_m and $1/CL$ values for five other phosphatidylcholines with a common MW identical with C(17):C(17)PC as shown in Table I. The temperature–axis intercepts and the slopes of these lines are replotted against ΔC (the insert B).

These two least-squares lines yield a unique pair of best-fit values of T_m and CL at the common point of intersection, and the coordinates of the intersection of the two lines (T_m = 133.0 °C and CL = 71.2 C–C bonds) are illustrated in Figure 2. Interestingly, this common temperature of 133.0 ± 1.3 °C is very close to the melting temperature of polyethylene at 138 °C (Nagle & Wilkinson, 1978). According to this figure, fully hydrated lamellae of C(72):C(72)PC and C(68):C(74)PC will exhibit calorimetrically the gel → liquid-crystalline phase transition at the virtually identical T_m of 133 °C. On the basis of two sets of DSC data of Lewis et al. (1987) and Wang et al. (1990), one can conclude that although the gel → liquid-crystalline phase behavior of phosphatidylcholines in the bilayer may be perturbed by the mismatch of the two acyl chains, the perturbation decreases progressively as the lipid acyl chain increases successively in chain length. Specifically, as the longer acyl chain reaches the effective chain length of 71.2 C–C bond lengths, the shorter acyl chain exerts no apparent effect on the phase transition temperature, provided that the apparent chain-length difference (ΔC) between the sn-1 and sn-2 acyl chains is somewhere in the neighborhood of 1.5–4.5 C–C bond lengths.

If we assume that the point of intersection of the two least-squares lines for C(X):C(X)PC and C(X):C(X+6)PC, shown in Figure 2A, defines the fixed coordinates of the apparent common point of intersection of lines drawn for other

Table I: Slopes and Vertical Intercepts of Various Straight Lines Connecting Coordinates of the Point of Intersection between the Two Least-Squares Line[a] for C(*X*):C(*X*)PC and C(*X*):C(*X*+6)PC (T_m = 133.0 °C and $1/CL$ = 0.014) and the T_m and $1/CL$ Values for Each of the Indicated Identical MW Phosphatidylcholines

phospholipids	ΔC	CL	$\Delta C/CL$	T_m °C[b]	slope	temperature-axis intercept
C(17):C(17)PC	1.5	16.0	0.094	49.0	−1733.565	157.348
C(15):C(19)PC	2.5	16.5	0.152	44.8	−1894.285	159.605
C(18):C(16)PC	3.5	17.0	0.206	44.4	−1978.624	160.790
C(14):C(20)PC	4.5	17.5	0.257	40.0	−2157.877	163.307
C(19):C(15)PC	5.5	18.0	0.306	39.0	−2264.481	164.805
C(13):C(21)PC	6.5	18.5	0.351	34.1	−2471.937	167.718
C(20):C(14)PC	7.5	19.0	0.395	33.2	−2586.388	169.326

[a] The two least-squares lines are $T_m = 157.23 - 1725.94\,(1/CL)$ for the C(*X*):C(*X*)PC series and $T_m = 163.41 - 2165.67\,(1/CL)$ for the C(*X*):C(*X*+6)PC series (Wang et al., 1990). [b] Data taken from Lin et al. (1990).

TOTAL CARBON ATOMS IN SN-1 ACYL CHAIN

	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
26										*0.380* **58.4**	*0.340* **62.1**	*0.300* **65.9**	*0.260* **69.6**	*0.220* **73.3**	*0.180* **77.0**	*0.140* **80.7**	*0.100* **84.4**	
25									*0.396* **53.7**	*0.354* **57.6**	*0.313* **61.6**	*0.271* **65.5**	*0.229* **69.5**	*0.188* **73.4**	*0.146* **77.4**	*0.104* **81.3**		
24									*0.370* **52.7**	*0.326* **56.9**	*0.283* **61.1**	*0.239* **65.3**	*0.196* **69.5**	*0.152* **73.7**	*0.109* **77.9**			
23								*0.386* **47.3**	*0.341* **51.8**	*0.296* **56.3**	*0.250* **60.8**	*0.205* **65.2**	*0.159* **69.7**	*0.114* **74.2**				
22								*0.357* **46.2**	*0.310* **51.0**	*0.262* **55.8**	*0.214* **60.6**	*0.167* **65.4**	*0.119* **70.2**					*0.106* **79.6**
21							*0.375* **40.0**	*0.325* **45.2**	*0.275* **50.3**	*0.225* **55.4**	*0.175* **60.6**	*0.125* **65.7**					*0.111* **76.1**	*0.149* **75.6**
20						*0.395* **33.2**	*0.342* **38.7**	*0.290* **44.3**	*0.237* **49.8**	*0.184* **55.3**	*0.132* **60.8**					*0.116* **72.2**	*0.156* **71.8**	*0.192* **71.5**
19						*0.361* **31.6**	*0.306* **37.5**	*0.250* **43.5**	*0.194* **49.4**	*0.139* **55.3**					*0.122* **68.0**	*0.163* **67.6**	*0.200* **67.4**	*0.234* **67.4**
18					*0.382* **23.6**	*0.324* **30.0**	*0.265* **36.4**	*0.206* **42.8**	*0.147* **49.2**					*0.128* **63.3**	*0.171* **63.0**	*0.209* **63.0**	*0.244* **63.1**	*0.277* **63.3**
17					*0.344* **21.6**	*0.281* **28.5**	*0.219* **35.4**	*0.156* **42.4**	*0.094* **49.3**				*0.135* **58.1**	*0.180* **58.0**	*0.220* **58.1**	*0.256* **58.3**	*0.289* **58.7**	*0.319* **59.3**
16				*0.367* **12.0**	*0.300* **19.5**	*0.233* **27.1**	*0.167* **34.6**	*0.100* **42.1**			*0.091* **52.6**	*0.143* **52.4**	*0.189* **52.4**	*0.231* **52.7**	*0.268* **53.1**	*0.302* **53.7**	*0.333* **54.4**	*0.362* **55.2**
15			*0.393* **1.1**	*0.321* **9.3**	*0.250* **17.5**	*0.179* **25.7**	*0.107* **33.9**			*0.097* **45.8**	*0.152* **45.9**	*0.200* **46.3**	*0.243* **46.7**	*0.282* **47.4**	*0.317* **48.1**	*0.349* **49.0**	*0.378* **50.0**	
14			*0.346* **-2.6**	*0.296* **6.4**	*0.192* **15.4**	*0.115* **24.4**			*0.103* **38.1**	*0.161* **38.6**	*0.212* **39.2**	*0.257* **40.1**	*0.297* **41.0**	*0.233* **42.0**	*0.366* **43.2**	*0.395* **44.4**		
13		*0.375* **-16.4**	*0.292* **-6.5**	*0.208* **3.4**	*0.125* **13.3**			*0.111* **29.3**	*0.172* **30.3**	*0.226* **31.4**	*0.273* **32.6**	*0.314* **33.9**	*0.351* **35.3**	*0.385* **36.7**				
12		*0.318* **-21.7**	*0.227* **-10.8**	*0.136* **0.2**			*0.120* **19.1**	*0.185* **20.7**	*0.241* **22.4**	*0.290* **24.2**	*0.333* **25.9**	*0.371* **27.7**						
11	*0.350* **-40.0**	*0.250* **-27.8**	*0.150* **-15.5**			*0.130* **7.1**	*0.200* **9.6**	*0.259* **12.1**	*0.310* **14.6**	*0.355* **17.0**	*0.394* **19.3**							
10	*0.278* **-48.5**	*0.167* **-34.7**			*0.143* **-7.3**	*0.217* **-3.4**	*0.280* **0.2**	*0.333* **3.6**	*0.379* **6.7**									
9	*0.188* **-58.6**			*0.158* **-24.6**	*0.238* **-18.8**	*0.304* **-13.8**	*0.360* **-9.2**											

TOTAL CARBON ATOMS IN SN-2 ACYL CHAIN

FIGURE 3: Calculated T_m values for 163 molecular species of saturated diacylphosphatidylcholines. The *X* and *Y* values in C(*X*):C(*Y*)PC are illustrated on the vertical and horizontal axes, respectively. The values of $\Delta C/CL$ and T_m are shown in a square box which represents a defined molecular species of phosphatidylcholine. The $\Delta C/CL$ and T_m values are printed in italic and boldface, respectively. Note that T_m values are calculated only for phosphatidylcholines with $\Delta C/CL$ values in the range of 0.09–0.40 based on eq 1.

homologous series of mixed-chain phosphatidylcholines with constant values of ΔC in the neighborhood of 4.5, then this fixed point provides a pair of apparent values of T_m and $1/CL$ which can be connected to other limited experimental data to yield a family of lines. As shown earlier in Figure 1, the T_m values of C(17):C(17)PC, C(15):C(19)PC, C(18):C(16)PC, C(14):C(20)PC, C(19):C(15)PC, C(13):C(21)PC, and C(20):C(14)PC are linearly related to $\Delta C/CL$, and the values of ΔC for this series of common MW phosphatidylcholines are in the range of 1.5–7.5 (Table I). Each of the data sets (T_m, $1/CL$) can now be connected to the assumed common point of intersection, and the resulting family of lines are graphically illustrated in Figure 2A. The slopes and vertical (temperature axis) intercepts of these lines are summarized in Table I. In addition, the slopes and the vertical intercepts are replotted aginst the value of ΔC (Figure 2B). The secondary plot of the slope value versus ΔC yields a straight line with a new slope, S_s (slope of slopes), of −142.844 ± 5.281 (SD) and a vertical intercept, I_s (intercept of slopes), of −1512.511 ± 26.003 (SD); similarly, the secondary plot of the original vertical intercept versus ΔC yields a straight line with a slope S_I (slopes of intercepts) of 2.006 ± 0.074 (SD) and a new vertical intercept I_I (intercept of intercepts) of 154.243 ± 0.365 (SD). The values of these final slopes and vertical intercepts are related to the coefficients of the empirical linear equation of T_m as:

$$T_m = S(1/CL) + I = [S_s(\Delta C) + I_s](1/CL) + [S_I(\Delta C) + I_I] = 154.2 + 2.0(\Delta C) - 142.8(\Delta C/CL) - 1512.5(1/CL) \tag{1}$$

This general expression enables the values of T_m to be calculated for fully hydrated saturated diacylphosphatidylcholines. Since eq 1 is derived empirically based on the existing data for fully hydrated phosphatidylcholines with values of $\Delta C/CL$ in the range of 0.09–0.40, this equation should be employed only to estimate the T_m values for those lipids with $\Delta C/CL$ values in the same range of 0.09–0.40.

Results and Discussion

As shown in Figure 3, the T_m values of fully hydrated bilayers for 163 molecular species of saturated diacylphosphatidylcholines are presented. These values were cal-

Table II: Comparison between Calculated and Experimental T_m Values for Saturated Phosphatidylcholines with $\Delta C/CL$ Values in the Range of 0.09–0.40

phospholipid	calculated T_m (°C)	observed T_m (°C)	% difference	ref
C(12):C(18)PC	24.2	23.4	−3.3	*a*
C(14):C(14)PC	24.4	23.9	−2.1	*b*
C(14):C(14)PC	24.4	24.2	−0.8	*c*
C(16):C(14)PC	27.1	27.2	+0.4	*d*
C(18):C(14)PC	30.0	29.9	−0.3	*a*
C(18):C(14)PC	30.0	31.6	+5.3	*d*
C(18):C(14)PC	30.0	29.8	−0.7	*e*
C(18):C(15)PC	36.4	38.1	+4.7	*g*
C(14):C(18)PC	38.6	38.2	−1.0	*a*
C(14):C(18)PC	38.6	40.3	+4.4	*d*
C(16):C(16)PC	42.1	41.4	−1.7	*b*
C(16):C(16)PC	42.1	41.6	−1.2	*c*
C(18):C(16)PC	42.8	44.4	+3.7	*f*
C(18):C(16)PC	42.8	44.9	+4.9	*a*
C(18):C(16)PC	42.8	45.0	+5.1	*d*
C(18):C(24)PC	63.0	62.8	−0.3	*g*
C(18):C(26)PC	63.3	63.9	+0.9	*g*

[a] Mattai et al. (1987). [b] Mabrey & Sturtevant (1976). [c] Singer et al. (1990). [d] Keough (1986). [e] Huang & Mason (1986). [f] Lin et al. (1990). [g] Unpublished work form this laboratory.

culated based on eq 1 for phosphatidylcholines whose *sn*-1 and *sn*-2 acyl chains are chosen to vary from 9 to 26 carbons and whose values of normalized chain-length difference ($\Delta C/CL$) are restricted within the range of 0.09–0.40. Several interesting features indicated by Figure 3 are worth notice: (1) For C(X):C(Y)PC with a constant value of X, the calculated T_m value is observed to increase markedly and linearly with increasing Y up to $Y = X - 1$ or X; thereafter, the increase in the T_m value is either small ($X < 16$) or negligible ($X \geq 16$). (2) For C(X):C(Y)PC with a constant value of Y, the calculated T_m value also increases linearly and markedly with increasing X up to $X = Y - 4$ or $Y - 3$; thereafter, the T_m value remains relatively constant ($Y \geq 17$) or increases only slightly ($Y < 17$). (3) The calculated T_m value increases continually as both X and Y in C(X):C(Y)PC increase simultaneously; however, the increase in the T_m value follows a function of rectangular hyperbola. This is observed in those diagonal terms in Figure 3 as shown, for example, by the continuous increase in the T_m value from −48.5 °C for C(10):C(9)PC to +84.4 °C for C(26):C(25)PC.

The calculated T_m values for some phospholipids are compared with the experimental data in Table II. Although the available experimental data for the lipids tested are rather limited, the agreements between the calculated and observed T_m values for various phosphatidylcholines are very good for the highest percent difference between them is only 5.3% (Table II). In fact, the percent difference between the experimental values for a given lipid determined from two different laboratories can often be greater than 5.3%. For instance, the observed T_m values for C(18):C(14)PC and for C(14):C(18)PC shown in Table II lead to the percent difference of 5.7 and 5.5%, respectively, based on the experimental data for these lipids. In addition, a reasonable correlation can be further detected between the calculated and experimental data as one examines the change in the T_m value for the homologous series of C(18):C(Y)PC. According to Figure 3, the calculated T_m value of C(18):C(Y)PC increases linearly and markedly as Y increases stepwise from 13 to 17; however, it remains relatively constant as Y increases beyond 21. The observed T_m values for the limited members of C(18):C(Y)PC shown in Table II indeed display this characteristic trend. This gives additional credence to our empirical approach in applying eq 1 to estimate the T_m values for various saturated diacylphosphatidylcholines with $\Delta C/CL$ values in the range of 0.09–0.40.

Although eq 1 of $T_m = 154.2 + 2.0(\Delta C) - 142.8(\Delta C/CL) - 1512.5(1/CL)$ has four terms with three variables (ΔC, $\Delta C/CL$, and $1/CL$), this empirical equation can be reduced to simpler forms under certain boundary conditions. First, when the apparent chain-length difference (ΔC) is held constant, the second term becomes the product of two constants which can be combined with the first term to yield a new constant, a_0. Similarly, the third term is converted to a function of $1/CL$ as ΔC is held constant. The resulting term can be combined with the fourth term to yield $b_0\ (1/CL)$, where b_0 is a new constant. Consequently, eq 1 is reduced to the form $T_m = a_0 + b_0(1/CL)$. In this special case (ΔC = constant), the T_m value is a simple linear function of $1/CL$. The two nearly vertical lines illustrated in Figure 1 can be regarded as examples of this special case. Second, for a series of phosphatidylcholines with identical MW, the difference between the value of $2CL$ and the value of ΔC is a constant, which corresponds to the thickness of the hydrocarbon region of the gel-state bilayer. In the series of phosphatidylcholines with a common MW identical with C(17):C(17)PC, the difference between $2CL$ and ΔC for various lipids is 30.5 C–C bond lengths (Table I). Consequently, eq 1 can be reduced to the form $T_m = 55.0 + 2.0(\Delta C) - 93.2(\Delta C/CL)$. Table I also gives the values of ΔC and $\Delta C/CL$ for this series of lipids; it can be shown by the least-squares analysis that $\Delta C = 19.919(\Delta C/CL) - 0.5096$. Substituting this linear relationship between ΔC and $\Delta C/CL$ into the above reduced function of T_m yields $T_m = 54.0 - 53.4(\Delta C/CL)$. Clearly, for this series of phospholipids with a common MW identical with C(17):C(17)PC, eq 1 can be reduced to a simple expression in which the T_m value is a linear function of $\Delta C/CL$ only. The nearly horizontal line illustrated in Figure 1 is an example of this special case. Finally, we should discuss one more special case in which the second and third terms of eq 1 are assigned to cancel out or $2.0(\Delta C) = 142.8(\Delta C/CL)$. To fulfill this boundary condition, CL must be 142.8/2 or 71.4, and the corresponding value of T_m is then a fixed temperature of 133.0 °C. These values of CL and T_m are in fact the coordinates of the common point of intersection of the lines drawn in Figure 2. At this point, the perturbational effects of ΔC and $\Delta C/CL$ on the chain melting are zero, because the two terms in eq 1 involving ΔC and $\Delta C/CL$ are canceled out in this special case.

In summary, we have derived empirically a general expression of $T_m = 154.2 + 2.0(\Delta C) - 142.8(\Delta C/CL) - 1512.5(1/CL)$ in which two apparent structural parameters, ΔC and CL, of a given phosphatidylcholine molecule and their ratio, $\Delta C/CL$, are used to specify the main phase transition temperature, T_m, of the given phosphatidylcholine bilayer in excess water. This empirical equation is employed in calculations of the T_m values of bilayers for 163 molecular species of saturated diacylphosphatidylcholines. The agreements between the calculated and observed T_m values are good with 5.3% as the largest percent difference for the lipids tested. It should be emphasized, however, that the calculated T_m value presented in this study should be used primarily as an estimate of the gel → liquid-crystalline phase transition temperature for designing experiments. When more experimental T_m values of mixed-chain phosphatidylcholines become available in the future, this empirical equation should be tested, refined, and improved continually. It is hoped that our empirical approach will eventually lead to the precise prediction of T_m values for a wide variety of fully hydrated phospholipids.

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Registry No. C(17):C(17)PC, 70897-27-7; C(15):C(19)PC, 127856-36-4; C(18):C(16)PC, 59403-53-1; C(14):C(20)PC, 122403-77-4; C(19):C(15)PC, 127856-37-5; C(13):C(21)PC, 127856-39-7; C(20):C(14)PC, 127856-38-6; C(12):C(18)PC, 127912-48-5; C(14):C(14)PC, 18194-24-6; C(16):C(14)PC, 69441-09-4; C(18):C(14)PC, 20664-02-2; C(18):C(15)PC, 130612-50-9; C(14):C(18)PC, 76343-22-1; C(16):C(16)PC, 63-89-8; C(18):C(16)PC, 59403-53-1; C(18):C(24)PC, 127856-46-6; C(18):C(26)PC, 130612-51-0.

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