brief communication

Analysis of the bilayer phase transition temperatures of phosphatidylcholines with mixed chains

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ABSTRACT The analysis of the chain-length dependence of the chain-melting transition temperatures of bilayers composed of lipids with identical chains (Marsh, D. 1991. *Biochim. Biophys. Acta.* 1062:1–6) is extended to include lipids with chains of unequal length. The bilayer transition temperatures of saturated asymmetrical phosphatidylcholines are interpreted by assuming that the transition enthalpy and transition entropy are linearly related to the absolute value of the difference in chain length between the *sn*-1 and *sn*-2 chains, with constant end contributions. Such an assumption is supported by calorimetric data on phosphatidylcholines of constant mean chainlength and varying chain asymmetry. In particular, a symmetrical linear dependence is observed on the chain asymmetry, Δn, which is centered around a value $\Delta n°$ that corresponds to the conformational inequivalence of the *sn*-1 and *sn*-2 chains. The transition temperature then takes the form: $T_t = T_t^{\infty}(n - n_H - h'|\Delta n + \Delta n°|)/(n - n_s - s'|\Delta n + \Delta n°|)$ where n_H, n_s are the end contributions, and h', s' are fractional deficits in the incremental transition enthalpy and entropy, respectively, arising from the overlapping regions of the longer chains. Optimization on the transition temperature data for the dependence on chain asymmetry of three series of phosphatidylcholines with constant mean chainlength, n, yields parameters that are capable of predicting the dependence of the transition temperatures on chain asymmetry for other mean chainlengths. The dependence of the transition temperature on mean chainlength for phosphatidylcholines in which the chain asymmetry is maintained constant, as well as the dependence on both mean chain length and chain asymmetry for phosphatidylcholines in which one of the two chains is maintained of constant length, are also described with high accuracy by using the same parameters.

INTRODUCTION

Bilayers composed of phospholipids with mixed chains of unequal length are of particular interest because of the known positional asymmetry in the chain composition of the phospholipids from biological membranes. Considerable insight can be gained into the physical properties of such bilayers by a study of the chain-melting transition temperatures (Huang, 1990). A prerequisite for a detailed description of the thermodynamics of the phase transition is, however, a knowledge of the dependence of the transition enthalpy and entropy on the lipid structure as reflected in the transition temperature (see, e.g., Cevc and Marsh, 1987).

In previous work (Marsh, 1991), such a relationship was established in an analysis of the chain-length dependence of the transition temperatures for bilayers composed of lipids with chains of equal length. The recent availability of extensive calorimetric data on phosphatidylcholines of the same mean chainlength but with differing chain asymmetries (Lin et al., 1990, 1991; Bultmann et al., 1991) now offers the possibility for a corresponding analysis of the dependence on the asymmetry in chain length.

The analysis is confined to phosphatidylcholines for which the chain asymmetry restricts the chain interdigitation in the gel phase to be only partial, rather than the full interdigitation found in the mixed interdigitated gel phase of phosphatidylcholines with one chain twice as long as the other (McIntosh et al., 1984; Hui et al., 1984; Mattai et al., 1987). In the partially interdigitated gel phase, the lipid chains pack in a manner more similar to that in the gel phases of lipids with symmetrical chains and overlap is restricted to the longer of the two chains from lipids in opposite sides of the bilayer (Huang, 1990). Such a structure might be expected to give rise to a deficit in the incremental transition enthalpy and entropy per CH_2 group because of the modified packing in the overlapping chain region. It is this principle that is applied here in the analysis of the dependence of the transition temperature on chain asymmetry.

An expression is derived that describes successfully the dependence of the transition temperature on both chain asymmetry and mean chain length in a manner that is consistent with the underlying thermodynamics of the phase transition. Previously, an empirical relation has been proposed by Huang (1991) that is able to

Abbreviations used in this paper: (n_1:0), (n_2:0)-PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine with saturated *sn*-1 chain of n_1 *C*-atoms and saturated *sn*-2 chain of n_2 *C*-atoms.

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predict the transition temperatures of both asymmetrical and symmetrical phosphatidylcholines with a high degree of accuracy. In this approach, the transition temperature is parameterized in terms of the effective chain asymmetry, ΔC, and the effective chain length, CL, and is restricted to values of $\Delta C/CL$ that are consistent with the formation of a partially interdigitated gel phase (Huang, 1990). Additionally, a perturbative approach has been offered by Cevc (1991) that also emphasizes the dependence on the chain asymmetry. One aim of the present work is to provide a thermodynamic understanding of the highly successful empirical expression obtained by Huang (1991).

THEORETICAL BACKGROUND

In a previous paper (Marsh, 1991), the chain-length dependences of the gel-to-fluid bilayer transition temperatures for lipids with a constant asymmetry between the two chains were interpreted in terms of a linear dependence of the transition enthalpy, ΔH_t, and the transition entropy, ΔS_t, on the mean chain length, n:

$$\Delta H_t = \Delta H_{inc}(n - n_0) \quad (1)$$

$$\Delta S_t = \Delta S_{inc}(n - n_0'), \quad (2)$$

where ΔH_{inc} and ΔS_{inc} are the incremental transition enthalpy and entropy, respectively, per $2CH_2$ groups, and n_0 and n_0' represent constant end contributions. This approximate linear dependence was supported by the results of calorimetric measurements and was found to be rather successful in describing the chain length dependence of the transition temperatures.

For lipids with chains of constant mean length but differing asymmetries, it might be anticipated that the transition enthalpy and entropy will be linearly related to the absolute values of the difference in length, $\Delta n = n_1 - n_2$, between the *sn*-1 and *sn*-2 chains. Because of the inherent conformational asymmetry between the two chains at the level of the glycerol backbone (Pearson and Pascher, 1979; Zaccai et al., 1979) the dependence on Δn will be offset by an amount $\Delta n°$ whose value is ~1.5 CH_2 units (Mason et al., 1981). This suggests that the dependence of the transition enthalpy and transition entropy, respectively, can be depicted by equations of the form:

$$\Delta H_t = \Delta H_{max} - \Delta h|n_1 - n_2 + \Delta n°| \quad (3)$$

$$\Delta S_t = \Delta S_{max} - \Delta s|n_1 - n_2 + \Delta n°|, \quad (4)$$

where $\Delta n°$ is the chain-length asymmetry at which ΔH_t and ΔS_t achieve their maximum values, that are given by ΔH_{max} and ΔS_{max}, respectively. Here, Δh and Δs can be interpreted as being the deficits in the incremental transition enthalpy and entropy, respectively, contributed by those sections of the chain which do not overlap within the same lipid molecule. The values of ΔH_{max} and ΔS_{max} are expected to be dependent on the (constant) mean chain length, as indicated by Eqs. 1 and 2.

An experimental justification of the functional form of Eqs. 3 and 4 is offered by the calorimetric data for phosphatidylcholines with constant mean chain lengths of $n = \frac{1}{2}(n_1 + n_2) = 17$, 15, and 14 that were obtained by Lin et al. (1990, 1991) and are given in Fig. 1. For each

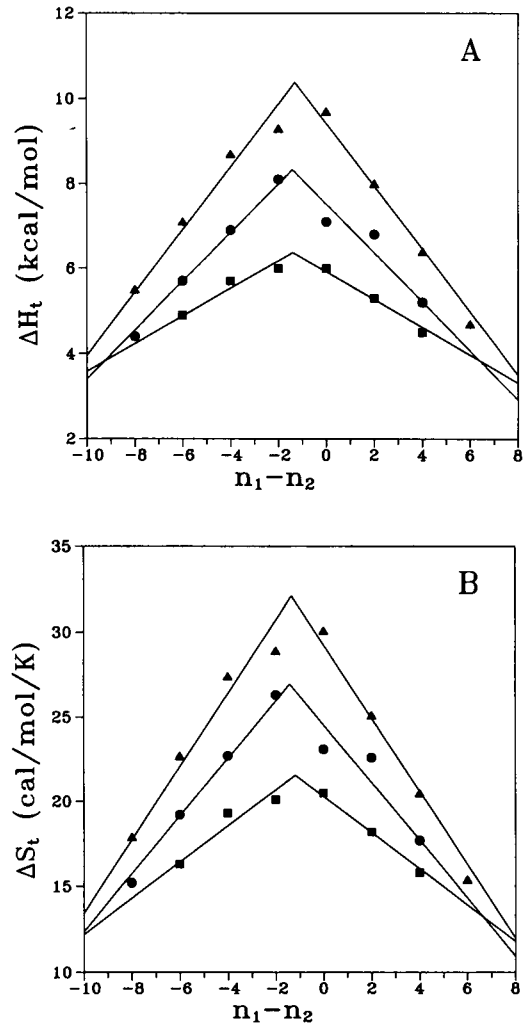

FIGURE 1 Dependence of (*A*) the transition enthalpy, ΔH_t, and (*B*) the transition entropy, ΔS_t, for bilayers of asymmetric phosphatidylcholines, (n_1:0), (n_2:0)-PC, on the difference in chain length, $n_1 - n_2$, between the *sn*-1 and *sn*-2 chains. Data are shown for phosphatidylcholines in which the mean chainlength $\frac{1}{2}(n_1 + n_2)$ is maintained constant at: 17 (▲), 15 (●) and 14 (■) *C*-atoms, and are taken from Lin et al. (1990, 1991). Full lines represent least squares fits to Eqs. 3 and 4, with the fitting parameters given in the text.

of the three series, the dependences of the transition enthalpies and entropies on the chain-length asymmetry, $n_1 - n_2$, are symmetrical about the same point, which differs from zero, and additionally they are approximately linear. These results correspond very well with the predictions of Eqs. 3, 4 and the maximum values of the calorimetric quantities increase with increasing mean chain length, in general accordance with Eqs. 1, 2.

A least squares fit of the calorimetric data in Fig. 1 to Eqs. 3 and 4 yields values of: ΔH_{max} = 10.4, 8.3, and 6.4 kcal/mol; Δh = 0.74, 0.56, 0.32 kcal/mol; with $\Delta n°$ = 1.3, 1.4, and 1.4; and ΔS_{max} = 32, 27, and 22 cal/mol/K; Δs = 2.2, 1.7, and 1.1 cal/mol/K; with $\Delta n°$ = 1.3, 1.4, and 1.2; for the asymmetric phosphatidylcholines with mean chain lengths n = 17, 15, and 14, respectively. The values of $\Delta n°$ (corresponding to the conformational bend at the beginning of the *sn*-2 chain) lie consistently within the range of 1.2–1.4 CH_2 groups which is close to the approximate value of 1.5 CH_2 units obtained by neutron diffraction for gel phase dipalmitoyl phosphatidylcholine (Zaccai et al., 1979). The incremental values, Δh and Δs, appear to change systematically with the mean chain length, but the precision of the data and the limited range of the data points are insufficient to be certain on this point. Interestingly, the ratio $\Delta h/\Delta s$ remains reasonably constant, as do the ratios $\Delta h/\Delta H_{max}$ and $\Delta s/\Delta S_{max}$ (at least for n = 17 and 15).

Combination of Eqs. 1 and 3, and Eqs. 2 and 4 suggests the following expressions for the dependence of the transition enthalpy and transition entropy on both the mean length, n, and asymmetry, Δn, of the lipid chains:

$$\Delta H_t = \Delta H_{inc}(n - n_H - h'|\Delta n + \Delta n°|) \quad (5)$$

$$\Delta S_t = \Delta S_{inc}(n - n_S - s'|\Delta n + \Delta n°|), \quad (6)$$

where n_H and n_S are the end contributions to the transition enthalpy and entropy, respectively, that are not included in the chain asymmetry. The reduced quantities $h' = \Delta h/\Delta H_{inc}$ and $s' = \Delta s/\Delta S_{inc}$ are the fractional deficits in the incremental transition enthalpy and entropy, respectively, for those sections of the chain that do not have intramolecular overlap.

The transition temperature can be defined in terms of the transition enthalpy and transition entropy by assuming that the chain-melting transition is essentially first order. In this case, the free energy change at the phase transition is zero ($\Delta G_t = \Delta H_t - T_t\Delta S_t = 0$) and the transition temperature is given by (cf Cevc and Marsh, 1987):

$$T_t = \Delta H_t/\Delta S_t = (\Delta H_{inc}/\Delta S_{inc})(n - n_H - h'|\Delta n + \Delta n°|)/(n - n_S - s'|\Delta n + \Delta n°|). \quad (7)$$

Eq. 7 characterizes the dependence of the transition temperature on both the mean length, n, and asymmetry, Δn, of the lipid chains. It may be used to fit the experimental chain-melting transition temperature data for different lipid bilayers if it is assumed that the quantities: $\Delta H_{inc}/\Delta S_{inc}$, n_H, n_S, $\Delta n°$, h'/s' and s' are essentially constant. The experimental data reviewed above suggest that, at least in part, this is in fact the case. This assumption is tested below by fitting the transition temperature data, which are inherently of higher precision than that of the calorimetric values that were considered above.

RESULTS AND DISCUSSION

The temperatures of the chain-melting transition for phosphatidylcholine bilayers composed of lipids with different chain asymmetries, but constant mean chain lengths of n = 17, 16, 15, and 14 are given in Fig. 2. The combined data sets for n = 17, 15 and 14 have been fitted to Eq. 7 by nonlinear least squares optimization, excluding only two data points that were found to give atypically large residuals. It is clear that the functional form of Eq. 7 is capable of describing the dependence on

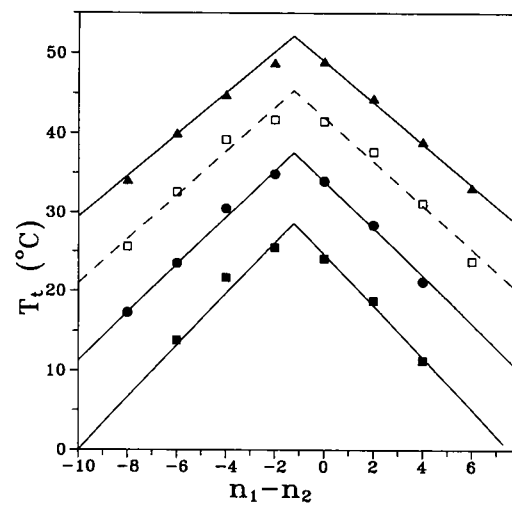

FIGURE 2 Dependence of the bilayer transition temperatures of asymmetric phosphatidylcholines, (n_1:0), (n_2:0)-PC, on the difference in chainlength, $n_1 - n_2$, between the *sn*-1 and *sn*-2 chains. Data are shown for phosphatidylcholines in which the mean chainlength $\frac{1}{2}(n_1 + n_2)$ is maintained constant at: 17 (▲), 16 (□), 15 (●), and 14 (■) *C*-atoms, and are taken from Lin et al., (1990; 1991) and Bultmann et al. (1991). The full lines represent a nonlinear least squares fit of the combined data sets for $\frac{1}{2}(n_1 + n_2)$ = 17, 15, and 14, excluding only (16:0), (18:0)-PC and (12:0), (16:0)-PC, to Eq. 7 with the parameters given in the text. The dashed line represents the dependence predicted for $\frac{1}{2}(n_1 + n_2)$ = 16 with these parameters.

n and Δn with a reasonably high degree of accuracy (rms = 0.5 K, $N = 19$). The values obtained for the fitting parameters are: $\Delta H_{inc}/\Delta S_{inc} = 426.4$ K, $n_H = 4.89$, $n_s = 1.12$, $\Delta n° = 1.24$, $h' = 0.117$ and $s' = 0.0287$. The value of $\Delta H_{inc}/\Delta S_{inc}$ is similar to those found previously in fitting the chain-length dependence for lipids with fixed chain asymmetry (Marsh, 1991) and the value of $\Delta n°$ is similar to that obtained from the calorimetric data and discussed above.

A first check on the general applicability of the values for the fitting parameters is offered by the data for n = 16 that is given in Fig. 2 but was not included in the optimization. It is seen that the same values of the parameters are able also to fit this data rather well. The transition temperatures in this data set all lie within ~1.5 K of the predicted curve and conform with the general trends for the other values of n. It will be noted further that all the predicted dependences of the transition temperatures on Δn are almost linear for a fixed value of n. This is in accord with the empirical observation by Huang and co-workers (see Huang, 1990) who found experimentally an approximately linear dependence on the normalized chainlength asymmetry parameter, $\Delta C/CL$.

A further test of the predictive power of Eq. 7 is given in Fig. 3 for two sets of data that were included only to a very limited extent in the optimization. It is seen that the dependence of the transition temperature on mean chain length for two extensive series of phosphatidylcholines in which the chain asymmetry is maintained constant either at $\Delta n = 0$ or at $\Delta n = -6$ is very well described by Eq. 7 without further adjustment of the fitting parameters. For the data set with $\Delta n = -6$, the agreement of the predicted dependence with the experimental data is essentially quantitative. None of the predicted values deviate from the experimental values by more than 0.6 K. For the data set with $\Delta n = 0$, the agreement is also rather good. With the exception of (12:0),(12:0)-PC, the predicted values are all within 1.1 K of the experimental ones.

Finally, the predictions of Eq. 7 are compared with the transition temperatures of phosphatidylcholines in which either the *sn*-1 or the *sn*-2 chain is maintained at a constant length of 18 *C*-atoms and the chain asymmetry (and mean chain length) is varied. As can be seen from Fig. 4, there is again good agreement between the experimental transition temperatures and the values predicted with the same parameters as those used in the other figures. With only one exception, the predicted values are within 0.5 K or less of the measured values. As pointed out by Huang (1991), the dependence of the transition temperature on chain asymmetry for lipids with one chain of fixed length is qualitatively similar to that for the lipids with constant mean chainlength, when the variable chain is shorter than the fixed chain, but

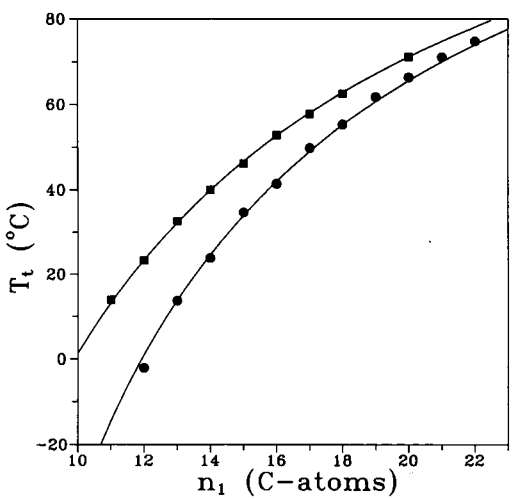

FIGURE 3 Dependence of the bilayer transition temperatures of phosphatidylcholines, (n_1:0), (n_2:0)-PC, on the length, n_1, of the *sn*-1 chain, where the length of the *sn*-2 chain is either $n_2 = n_1$ (●) or $n_2 = n_1 + 6$ (■). Data are from Lewis et al. (1987) and Wang et al. (1990), respectively. The full lines are the transition temperatures predicted according to Eq. 7, with the parameters obtained from fitting the data in Fig. 2.

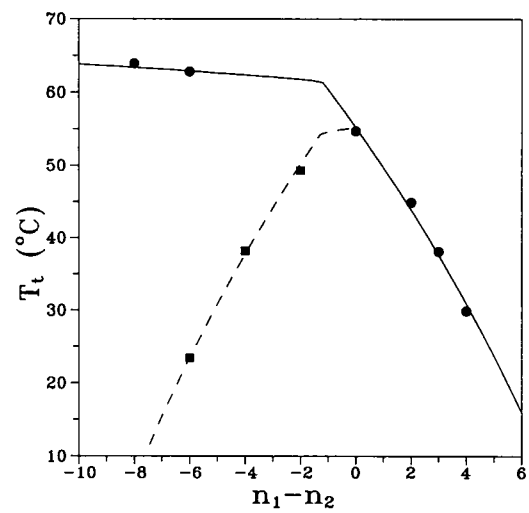

FIGURE 4 Dependence of the bilayer transition temperatures of asymmetric phosphatidylcholines, (n_1:0), (n_2:0)-PC, on the difference in chain length, $n_1 - n_2$, between the *sn*-1 and *sn*-2 chains. Data are shown for phosphatidylcholines in which either the *sn*-1 chainlength is maintained constant at n_1 = 18 *C*-atoms (●) or the *sn*-2 chainlength is maintained constant at n_2 = 18 *C*-atoms (■). Data are taken from Mattai et al. (1987) and Huang (1991). The full line is the dependence for n_1 = 18 and the dashed line that for n_2 = 18, as predicted by Eq. 7, with the parameters obtained by fitting the data in Fig. 2.

beyond this the dependence on the asymmetry is very small.

It is clear from the data presented in Figs. 2–4 that Eq. 7 with fixed parameters is capable of describing the chain-melting transition temperatures of saturated phosphatidylcholines over a wide range of chain length and chain asymmetry, with a high degree of accuracy. The expression for the transition temperature, Eq. 7, contains six parameters but the combined data set that can be described by these fixed parameters is rather large. Recently, Huang (1991) has derived an empirical equation which is capable of predicting the transition temperature data for a similar range of asymmetrical phosphatidylcholines to high accuracy. This equation contains four adjustable parameters which were fitted, and in addition a fixed offset of 1.5 *C*-atoms in the asymmetry parameter, ΔC, as well as an end correction to the chain length, *CL*, which depended on which of the two chains was the longer. A correlation of this expression with the present analysis can be made by expanding the denominator in Eq. 7 to first order. This yields the following approximation for the transition temperature:

$$T_t \approx (\Delta H_{inc}/\Delta S_{inc}) \times [1 - (n_H - n_S)/n - (h' - s')|\Delta n + \Delta n^\circ|/n]. \quad (8)$$

This approximation also holds if n is offset by a constant amount. With the exception of a term linear in the asymmetry parameter, ΔC, Eq. 8 has a form essentially identical to that given by Huang (1991). To this extent, the present analysis provides a thermodynamic justification for the extremely successful empirical fits provided by Huang (1991). The term linear in ΔC introduced in the latter work arises because the transition temperature was constrained to be the same for all lipids at a value of $CL = 71.2$, rather than at the limiting value of $n \rightarrow \infty$ as in the present treatment. Presumably the latter criterion is the physically more realistic.

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