Thermodynamic Analysis of Chain-Melting Transition Temperatures for Monounsaturated Phospholipid Membranes: Dependence on *cis*-Monoenoic Double Bond Position

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ABSTRACT Unsaturated phospholipid is the membrane component that is essential to the dynamic environment needed for biomembrane function. The dependence of the chain-melting transition temperature, T_t, of phospholipid bilayer membranes on the position, n_u, of the *cis* double bond in the glycerophospholipid *sn*-2 chain can be described by an expression of the form $T_t = T_t^c(1 + h'_c|n_u - n_c|)/(1 + s'_c|n_u - n_c|)$, where n_c is the chain position of the double bond corresponding to the minimum transition temperature, T_t^c, for constant diacyl lipid chain lengths. This implies that the incremental transition enthalpy (and entropy) contributed by the *sn*-2 chain is greater for whichever of the chain segments, above or below the double-bond position, is the longer. The critical position, n_c, of the double bond is offset from the center of the *sn*-2 chain by an approximately constant amount, $\delta n_c \approx 1.5$ C-atom units. The dependence of the parameters T_t^c, h'_c, and s'_c on *sn*-1 and *sn*-2 chain lengths can be interpreted consistently when allowance is made for the chain packing mismatch between the *sn*-1 and *sn*-2 chains. The length of the *sn*-2 chain is reduced by ~0.8 C-atom units by the *cis* double bond, in addition to a shortening by ~1.3 C-atom units by the bent configuration at the C-2 position. Based on this analysis, a general thermodynamic expression is proposed for the dependence of the chain-melting transition temperature on the position of the *cis* double bond and on the *sn*-1 and *sn*-2 chain lengths. The above treatment is restricted mostly to double-bond positions close to the center of the *sn*-2 chain. For double bonds positioned closer to the carboxyl or terminal methyl ends of the *sn*-2 chain, the effects on transition enthalpy can be considerably larger. They may be interpreted by the same formalism, but with different characteristic parameters, h'_c and s'_c, such that the shorter of the chain segments makes a considerably smaller contribution to the calorimetric properties of the chain-melting transition.

INTRODUCTION

Biological membranes are composed of lipids with medium to long hydrocarbon chains. This is needed to maintain an efficient permeability barrier for polar and ionic solutes and to provide adequate hydrophobic matching with the transmembrane spans of integrally anchored proteins. The major lipid components of most eukaryotic and prokaryotic membranes are normal diacyl glycerophospholipids. Such lipids with saturated chains of C-16 length and above would cause the membranes to be in a bilayer gel state at physiological temperatures, rather than in the fluid liquid-crystalline state that provides the dynamic environment essential to many membrane functions. In natural membranes, the fluid state is maintained by introducing *cis* double bonds into the *sn*-2 chains. A single double bond situated close to the center of the chain is most effective in lowering the chain-melting transition temperature of the bilayer membranes (Barton and Gunstone, 1975). It is *cis*-monoenoic fatty acyl chains of this type (i.e., oleic) that are predominant in mammalian cell membranes (see, e.g., Marsh, 1990).

Following the pioneering work of Barton and Gunstone (1975) on *cis*-bond positional isomers of dioctadecenoyl phosphatidylcholines, Huang and co-workers (Wang et al., 1994, 1995a,b; Huang et al., 1996) have undertaken extensive synthetic work, coupled with high-sensitivity calorimetric studies, on homologous series of both phosphatidylcholines and phosphatidylethanolamines with saturated *sn*-1 chains and *cis*-monoenoic *sn*-2 chains. This pattern of relative chain compositions corresponds to that found in natural phospholipid species. Systematic variations were made in the position of the *cis* double bond in the *sn*-2 chain, as well as in both *sn*-1 and *sn*-2 chain lengths. The aim of these studies was to delineate the structural features of the lipid molecules that contribute to lowering the chain-melting transition in biological membranes. Furthermore, molecular mechanics calculations were performed to deduce energetic and structural details of the relative packing of the *sn*-1 and *sn*-2 chains that is likely to obtain in the phospholipid gel phase (see also Li et al., 1994). From this it was concluded that the two C-C single bonds adjacent to the *cis* double bond are in a skew (120° dihedral) conformation and that, additionally, a neighboring *gauche* (65° dihedral) conformation produces a kink in the *sn*-2 chain that otherwise is aligned parallel with the *sn*-1 chain (Wang et al., 1994, 1995a,b). The resulting $g^+s^+\Delta s^+$ configuration divides the chain into a longer and a shorter segment, which were suggested to interact differently with the *sn*-1 chain (Huang et al., 1996; Li et al., 1998).

By using multiple regression analysis, Huang and co-workers (Wang et al., 1994, 1995a,b) have also derived various expressions that relate the transition temperature to

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structural parameters characterizing the bilayer thickness, the two segments of the *sn*-2 chain, and the mismatch between the *sn*-1 and *sn*-2 chains. These expressions have a high predictive capacity and emphasize the importance of the various structural parameters in determining the chain melting of unsaturated lipids. However, the functional forms of the equations are largely empirical, because they are optimized for fitting the experimental transition temperatures, rather than directly reflecting the underlying thermodynamics of chain melting.

The aim of the present paper is to interpret the transition temperatures in terms of the dependence of the chain-melting transition enthalpy and entropy on the structural parameters, particularly those determined by the position of the *cis* double bond in the *sn*-2 chain. Such an approach proved successful in the analysis of the transition temperatures of saturated mixed-chain lipids with varying mismatch between the lengths of the *sn*-1 and *sn*-2 chains (Marsh, 1992). In the present case, however, the dependence of the transition enthalpy on the position of the double bond is relatively small for chains of constant length. Therefore, the analysis must be led first by the dependence on the double-bond position of the transition temperatures, which can be defined with greater accuracy. Only then is it possible to construct a consistent thermodynamic model in terms of the structural parameters relating to the double-bond position. Finally, the thermodynamic effects of mismatch between the *sn*-1 and *sn*-2 chains may be added to the model to explore a wider range of chain lengths. In this way, the factors contributing to the lowering of the chain-melting transition of natural membrane phospholipids may be characterized thermodynamically in terms of the structural parameters of the lipid molecules. For the analysis, emphasis is placed on double-bond positions close to the center of the *sn*-2 chain, as is the situation for natural membrane lipids. For double bonds close to the chain ends, the effects can be quantitatively different.

DEPENDENCE OF PHOSPHATIDYLCHOLINE TRANSITION TEMPERATURES ON THE DOUBLE-BOND POSITION

Fig. 1 gives the dependence of the chain-melting transition temperature, T_t, on the position, n_u, of the single *cis* double bond in the C18:1 *sn*-2 acyl chain for aqueous bilayer dispersions of 1-acyl-2-(18:1cΔ^{n_u}) phosphatidylcholines (PCs). Data are given for five series of PC lipids with different *sn*-1 acyl chains. Only for one of the series is the *sn*-1 chain unsaturated and matched with the *sn*-2 chain. For the other series, the *sn*-1 chain is saturated, and its length remains constant within a given series. For all series, the transition temperatures fall to a minimum for a characteristic position of the *cis* double bond, $n_u = n_c$ (≈ 10) close to the center of the C18:1 *sn*-2 chain, and increase again as the double bond moves toward the terminal methyl end of the chain. The dependence of T_t on n_u is approximately symmetrical about the position $n_u = n_c$, for the PC series where there are sufficient data with $n_u > n_c$.

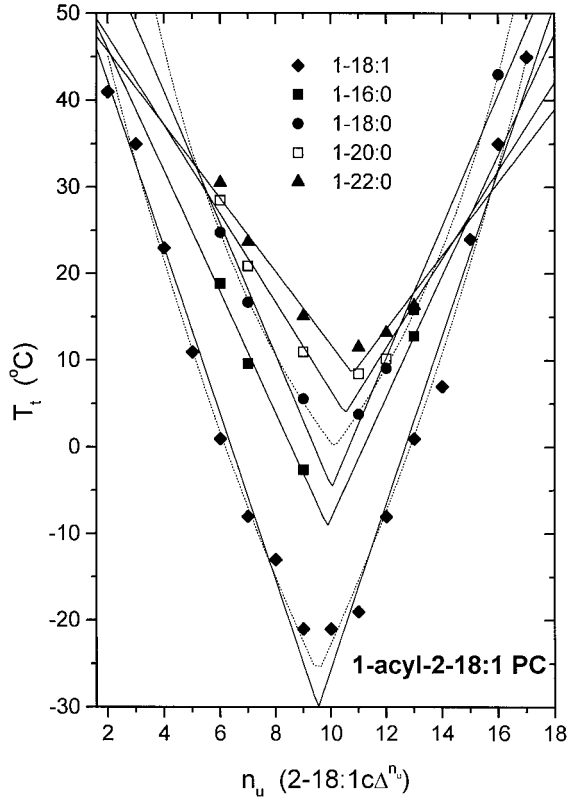


FIGURE 1 Dependence of the chain-melting transition temperature, T_t, of hydrated 1-acyl-2-(18:1cΔ^{n_u})-*sn*-glycero-3-phosphocholines on the position, n_u, of the *cis* double bond in the *sn*-2 chain. The *sn*-1 acyl chain is 18:1cΔ^{n_u} (◆), 16:0 (■), 18:0 (●), 20:0 (□), or 22:0 (▲). Data are from Barton and Gunstone (1975), Wang et al. (1995a,b), Huang et al. (1996), and Dekker et al. (1983). The solid lines are bilinear least-squares fits to the data of Eq. 3, with $s'_c = 0$ and the parameters given in Table 1. ·····, Nonlinear fit with s'_c unconstrained (for 1–18:1cΔ^{n_u}).

The overall size of the calorimetric enthalpies and the fact that no other high-enthalpy transitions are observed at higher temperatures demonstrate that all of the calorimetric transitions given in Fig. 1 correspond to the chain-melting event. A systematic thermodynamic analysis requires, in addition to this, that the lipid phases immediately above and below the chain melting (particularly those below) should be in thermodynamically similar states for a given lipid series. This is evidenced by the systematic trends in the transition temperatures, within a given series, and to a less stringent degree by those between different series. Thermodynamically significant differences in the phase identities should be reflected by discontinuities in the data, which are not seen. It cannot be excluded, however, that the low-temperature phases may differ between different lipid series, particularly between the diunsaturated and monounsaturated phosphatidylcholine series (cf. Lewis et al., 1988). (Structural characterizations of the phase transitions exhibited by most of the unsaturated lipids considered here have not been performed yet, and this could have an impact on the intercomparison between different series.)

The simplest empirical thermodynamic means of interpreting the biphasic behavior of the transition temperature seen in Fig. 1 is to assume that both the transition enthalpy, ΔH_t, and the transition entropy, ΔS_t, change linearly and symmetrically with distance of the double bond from the critical position, n_c:

$$\Delta H_t = \Delta H_t^c + \Delta h_c|n_u - n_c| \quad (1)$$

$$\Delta S_t = \Delta S_t^c + \Delta s_c|n_u - n_c| \quad (2)$$

where ΔH_t^c and ΔS_t^c are the values of ΔH_t and ΔS_t, respectively, for $n_u = n_c$. The thermodynamic implications of Eqs. 1 and 2 are that contributions to the calorimetric quantities differ for the sections of the *sn*-2 chain above and below the double bond, depending on which is the longer (see later section on the thermodynamic model). The transition temperature for a first-order transition ($\Delta G_t = \Delta H_t - T_t\Delta S_t = 0$) is given from Eqs. 1 and 2 by

$$T_t = T_t^c \frac{1 + h_c'|n_u - n_c|}{1 + s_c'|n_u - n_c|} \quad (3)$$

where T_t^c ($\equiv \Delta H_t^c/\Delta S_t^c$) is the value of the transition temperature for $n_u = n_c$, and $h_c' \equiv \Delta h_c/\Delta H_t^c$, $s_c' \equiv \Delta s_c/\Delta S_t^c$ are the normalized incremental positional deficits in the calorimetric quantities.

The transition enthalpies measured experimentally change relatively slowly with double-bond position for the PCs with saturated *sn*-1 chain (Wang et al., 1995a) and for the 1-(18:1cΔ^{n_u})-2-(18:1cΔ^{n_u}) PCs over the range $n_u = 5$–12 (Barton and Gunstone, 1975). The trend, however, is for ΔH_t to decrease as n_u approaches n_c, i.e., h_c' is positive. For the experimental transition entropies, the individual values do not differ greatly, and there is little obvious systematic dependence on the double-bond position for any of the PC series (Barton and Gunstone, 1975; Wang et al., 1995a,b). For the PCs with saturated *sn*-1 chains, the differences in ΔS_t between PCs with different n_u are mostly smaller than the individual error ranges (Wang et al., 1995a,b). A reasonable first approximation is therefore to assume that $s_c' \approx 0$ in fitting the transition temperatures.

Least-squares fits of Eq. 3 to the transition temperature data from the five series of PC lipids, with the constraint that $s_c' = 0$, are given in Fig. 1. The dependence of T_t on n_u can be described by Eq. 3 with a reasonable degree of precision for all five lipid series. Corresponding values of the fitting parameters are given in Table 1. The fits are overdetermined in all cases, although the number of data points with $n_u > n_c$ is relatively small for the PCs with C20:0 or C22:0 *sn*-1 chains, and particularly for the series with C16:0 *sn*-1 chain. As anticipated, the fitted values for n_c are all rather similar, lying between 9.5 and 10.8 for the C18:1 chains of the different PC series, but they do show a slight systematic trend with length and degree of unsaturation of the *sn*-1 acyl chain. The fitted values for the minimum transition temperature, T_t^c, show the expected trend, i.e., they increase systematically with degree of saturation and length of the *sn*-1 acyl chain.

The values for the fractional incremental transition enthalpies, h_c', given for the PC series in Table 1, are all positive and relatively small, as expected. The corresponding values of Δh_c are in the region of 0.1–0.2 kcal/mol/CH_2, which explains why the effects are not very readily measurable calorimetrically. For the PC series with saturated *sn*-1 chains, the absolute values of h_c' are consistent to within experimental error with fits of the experimental transition enthalpies (Wang et al., 1995b) according to Eq. 1. The values of ΔS_t^c, calculated from ΔH_t^c and the values of T_t^c from Table 1, are 22.3 ± 0.7, 23.6 ± 0.4, and 25.6 ± 0.6 cal · mol^{-1} · K^{-1} for $n_1 = 18$, 20, and 22, respectively, which are quite close to the mean values of the experimen-

TABLE 1 Parameters characterizing the dependence of the chain-melting transition temperatures, T_t, of hydrated 1-acyl-2-(18:1cΔ^{n_u})-*sn*-glycero-3-phosphocholines and -phosphoethanolamines on the position, n_u, of the *cis* double bond in the *sn*-2 chain, according to Eq. 3

sn-1	T_t^c (K)	n_c	h_c'	s_c'
Phosphatidylcholines				
18:1cΔ^{n_u}	243.1 ± 1.7	9.53 ± 0.09	0.039 ± 0.002	0.0*
	247.0 ± 1.8	9.51 ± 0.07	−0.008 ± 0.014	−0.036 ± 0.011
16:0	264.1 ± 2.4	9.87 ± 0.14	0.026 ± 0.003	0.0*
18:0	268.5 ± 1.8	10.02 ± 0.12	0.028 ± 0.002	0.0*
20:0	277.1 ± 1.8	10.51 ± 0.19	0.018 ± 0.003	0.0*
22:0	281.6 ± 2.0	10.78 ± 0.23	0.015 ± 0.003	0.0*
Phosphatidylethanolamines				
16:0	295.3 ± 1.6	9.97 ± 0.2	0.011 ± 0.002	0.0*
	295.4 ± 1.5	9.97 ± 0.2	0.0*	−0.011 ± 0.002
18:0	301.4 ± 0.4	10.20 ± 0.12	0.008 ± 0.001	0.0*
	301.4 ± 0.7	10.20 ± 0.12	0.0*	−0.008 ± 0.001
	300.0 ± 3.3	10.24 ± 0.15	0.21 ± 0.50	0.20 ± 0.48
20:0	304.2 ± 0.5	10.23 ± 0.11	0.0072 ± 0.0008	0.0*
	304.2 ± 0.5	10.23 ± 0.11	0.0*	−0.0070 ± 0.0008
22:0	304.8 ± 0.4	10.18 ± 0.08	0.0072 ± 0.0006	0.0*
	304.8 ± 0.4	10.18 ± 0.08	0.0*	−0.0070 ± 0.0006

*Fixed.

tally determined transition entropies 22.8 ± 1.2, 23.8 ± 0.6, and 25.8 ± 0.6 cal · mol^{-1} · K^{-1}, respectively, averaged over the range n_u = 6–13 (Wang et al., 1995a). The bilinear fits of the transition temperatures with $s'_c = 0$ that are given in Fig. 1 for the PCs with saturated *sn*-1 chains are therefore consistent with the available calorimetric data.

The situation for PC series with 18:1cΔ^{n_u} *sn*-1 chains is more complex because data are available over a wider range of values for n_u. This reflects itself in the fact that the bilinear fit with $s'_c = 0$, over the entire range of n_u values, deviates somewhat from the experimental data for values of n_u close to n_c. The larger data set allows nonlinear fitting with unrestricted s'_c, and the result is given by the dotted line in Fig. 1. The least-squares value of h'_c required is close to zero, and s'_c is negative (Table 1). The experimentally determined transition entropies do not show a systematic trend with n_u over the entire range, although there is a local maximum close to $n_u = n_c$ that is consistent with s'_c being negative (Barton and Gunstone, 1975). However, the experimental transition enthalpy increases considerably for the double bond close to either end of the *sn*-1 and *sn*-2 chains (Barton and Gunstone, 1975). It is therefore likely that a hybrid model is more appropriate in considering the full range of double bond positions (n_u = 2–17) for the 1-(18:1cΔ^{n_u})-2-(18:1cΔ^{n_u}) PCs (see later).

The chain-melting event exhibited by the diunsaturated 1-(18:1cΔ^9)-2-(18:1cΔ^9) PC has been shown to involve a transition from a lamellar crystalline-like phase to the lamellar liquid-crystalline phase, rather than from a lamellar gel phase (Lewis et al., 1988). Currently it is not known whether variations in the nature of this low-temperature phase might contribute to the rather complex behavior of the complete 1-(18:1cΔ^{n_u})-2-(18:1cΔ^{n_u}) PC series. It should also be noted that the calorimetric curves of certain of the monounsaturated phosphatidylcholines contain lower-enthalpy peaks at lower temperatures that represent ordered-phase transitions and, in some cases, possibly pretransitions (Wang et al., 1995a). This and the thermal history suggest rather strongly that chain-melting takes place from a gel phase for the monounsaturated lipids, although direct structural information is currently not available.

DEPENDENCE OF PHOSPHATIDYLETHANOLAMINE TRANSITION TEMPERATURES ON DOUBLE-BOND POSITION

Fig. 2 gives the dependence of the chain-melting transition temperature on double-bond position for analogous series of 1-acyl-2-(18:1cΔ^{n_u}) phosphatidylethanolamines (PEs) with saturated *sn*-1 acyl chains. These chain-melting events are assigned by Wang et al. (1994), on the basis of the thermal history, to lamellar gel-to-lamellar liquid-crystalline transitions. For the phosphatidylethanolamines, the sizes of the data sets are smaller than for the phosphatidylcholines, but bilinear fits with the constraint $s'_c = 0$ can be obtained in each case. For 1-(18:0)-2-(18:1cΔ^{n_u}) PE, an unrestricted fit

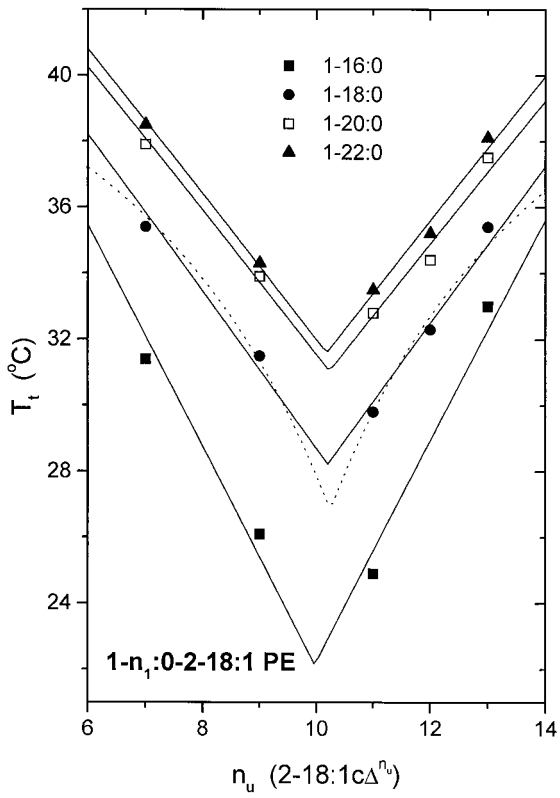


FIGURE 2 Dependence of the chain-melting transition temperature, T_t, of hydrated 1-acyl-2-(18:1cΔ^{n_u})-*sn*-glycero-3-phosphoethanolamines on the position, n_u, of the *cis* double bond in the *sn*-2 chain. The *sn*-1 acyl chain is 16:0 (■), 18:0 (●), 20:0 (□), or 22:0 (▲). Data are from Wang et al. (1994) and Huang et al. (1996). ——, Bilinear least-squares fits to the data of Eq. 3, with $s'_c = 0$ and the parameters given in Table 1. ·····, Nonlinear fit with s'_c unconstrained for 1-(18:0)-2-(18:1) PEs.

with Eq. 3 is also shown. The fitting parameters are given in Table 1. The fitted values of n_c are equal to within experimental error for the different PE series and lie within the same region as those obtained for the PC series with the same monounsaturated *sn*-2 acyl chains. Because the variations in transition temperature are relatively slight, and the values of h'_c are correspondingly small, equally good fits are obtained with fixed $h'_c = 0$ and varying s'_c. The resulting values of s'_c (Table 1) are of equal magnitude but of opposite sign to those of h'_c in the complementary fits. For PEs, it has been suggested that the effects of double-bond position are principally entropic (Wang et al., 1994), but the values of the calorimetric enthalpies and entropies do not vary systematically and therefore are not decisive on this point. Enthalpic contributions are favored here because this is evidenced by direct calorimetric measurements for the PC series (Barton and Gunstone, 1975; Wang et al., 1995b).

In contrast to the phosphatidylcholine series, the values for the fractional incremental enthalpy and entropy, h'_c and s'_c, are both positive for unrestricted fits with the 1-(18:0)-2-(18:1cΔ^{n_u}) PE series (Table 1). Correspondingly, the predicted dependence of T_t on n_u curves downward more steeply as n_u approaches n_c. The data points are too few and

the uncertainties in the h_c' and s_c' parameters are too great, however, to establish a significant difference between the PE and PC series in this respect. The experimentally measured transition enthalpies and entropies vary relatively little and show no systematic trend with n_u for any of the PE series (Wang et al., 1994). The calorimetric quantities therefore do not help to resolve this uncertainty, but are consistent with h_c' being considerably smaller for the PE series than for the PC series, in agreement with the values given in Table 1.

THERMODYNAMIC MODEL

Given the consistency of the above semiempirical analysis, it is possible to propose a specific thermodynamic model that is in agreement with the systematic trends in the data. This model is indicated schematically in Fig. 3. The sections of the unsaturated *sn*-2 chain above and below the *cis* double bond contribute differently to the calorimetric properties of the chain-melting transition (cf. Huang et al., 1996; Li et al., 1998). The longer of the two sections contributes an incremental enthalpy, $\Delta H_{inc,2}$, per methylene group that is greater by an amount Δh_c than that contributed by the shorter section of the chain. It is not necessarily assumed, as in one previous analysis (Cevc, 1991), that the shorter section of the chain does not contribute at all to the thermodynamics of chain melting, although this possibility is covered by the special case $\Delta h_c = \Delta H_{inc,2}$. Further, to allow for end effects in the two sections of the *sn*-2 chain, the section above the double bond is reduced in effective length by an amount δn_u and that below the double bond by an amount δn_2. If the upper Δ-section of the chain is the longer, then the calorimetric enthalpy is given by

$$\Delta H_t(n_u > n_c) = \Delta H_{inc,2}(n_u - \delta n_u) + (\Delta H_{inc,2} - \Delta h_c) \cdot (n_2 - n_u - \delta n_2) + \Delta H_o(n_1, n_2) \quad (4)$$

Correspondingly, when the lower ω-section of the chain is the longer,

$$\Delta H_t(n_u < n_c) = \Delta H_{inc,2}(n_2 - n_u - \delta n_2) + (\Delta H_{inc,2} - \Delta h_c) \cdot (n_u - \delta n_u) + \Delta H_o(n_1, n_2) \quad (5)$$

where n_2 is the total number of C-atoms in the *sn*-2 chain. (Counting of the unsaturated C-atoms is allowed for by the general end reductions, δn_u and δn_2.) In both Eqs. 4 and 5, $\Delta H_o(n_1,n_2)$ represents all other contributions to the calorimetric enthalpy, including those from the polar headgroup and *sn*-1 chain of length n_1. This additional contribution contains a term that depends on the length, n_2, of the *sn*-2 chain and arises from the mismatch in *sn*-1 and *sn*-2 chain packing at the chain ends (see Huang, 1990; Marsh, 1992; and later discussion).

Equations 4 and 5 define the model and may be expressed

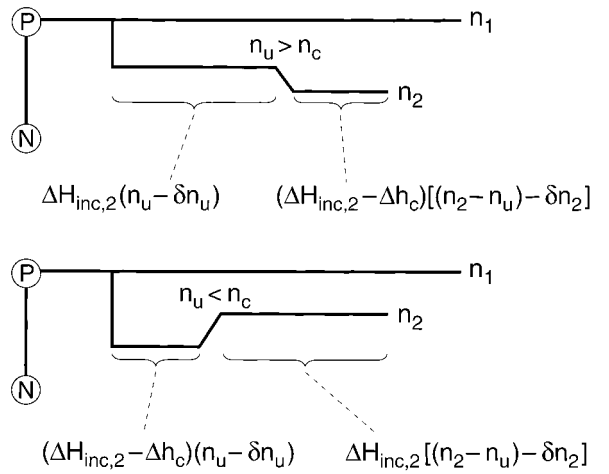


FIGURE 3 Schematic diagram of the chain packing in diacyl phospholipids with monounsaturated *sn*-2 chains (n_2:1cΔ^{n_u}). The incremental chain-melting transition enthalpies are $\Delta H_{inc,2}$ and $\Delta H_{inc,2}$-Δh_c per CH_2 group for the longer and shorter segments, respectively, of the *sn*-2 chain. The effective length of the upper Δ-segment of the *sn*-2 chain is n_u-δn_u, and that of the lower ω-segment is (n_2-n_u)-δn_2.

in a common form that is identical with Eq. 1, where

$$\Delta H_t^c = (\Delta H_{inc,2} - \Delta h_c/2)(n_2 - \delta n_2 - \delta n_u) + \Delta H_o(n_1, n_2) \quad (6)$$

and

$$n_c = n_2/2 + (\delta n_u - \delta n_2)/2 \quad (7)$$

Correspondingly, ΔS_t^c in Eq. 2 for the transition entropy is given by an expression analogous to Eq. 6. It is seen immediately from Eq. 7 that the critical position of the double bond, n_c, is offset from the center of the chain (n_2/2) by an amount $\delta n_c \equiv \frac{1}{2}(\delta n_u - \delta n_2)$, which has a value in the range ~0.9–1.8 for phospholipids with a saturated *sn*-1 chain (see Table 1). On the basis of molecular mechanics calculations, Huang and co-workers (Wang et al., 1994) have pointed out that $\delta n_u = 5$ and $\delta n_2 = 2$, when allowance is made for the $g^+s^+\Delta s^+$ configuration about the double bond and the bent configuration at the C-2 position of the *sn*-2 chain. The resulting prediction that $\delta n_c \approx 1.5$, according to Eq. 7, is therefore in good agreement with the experimental values deduced above from their calorimetric data.

If the net length of the *sn*-2 chain (after allowing for the shortening effect of the double bond and the bent conformation at the glycerol backbone) is shorter than that of the *sn*-1 chain, then the term $\Delta H_o(n_1, n_2)$ in Eq. 6 is linearly dependent on n_1 and n_2 (see Marsh, 1992). Without loss of generality, Eq. 6 then can be written as

$$\Delta H_t^c = \Delta H'_{inc,1} n_1 + \Delta H'_{inc,2} n_2 + \Delta H_o \quad (8)$$

which gives the explicit dependence on n_1 and n_2. This latter equation can be used to analyze further the parameters other than n_c that are deduced from Figs. 1 and 2. Again, an analogous equation applies to ΔS_t^c. Even the shortest *sn*-1

chain (C16:0) of these data sets is slightly longer than the 18:1c *sn*-2 chain, which is shortened by $\Delta n^{o} \approx 2.3$ C-atoms (Wang et al., 1995a) and therefore fulfills the above condition. When n_2 is constant, as is the case in Figs. 1 and 2, Eq. 8 can be further simplified as

$$\Delta H_t^c = \Delta H'_{inc,1}(n_1 - n_{1,o}) \tag{9}$$

where $n_{1,o}$ [$\equiv -(\Delta H_o + \Delta H'_{inc,2} n_2)/\Delta H'_{inc,1}$] represents all contributions that do not depend on n_1. The dependence of the minimum transition temperature, T_t^c, on the *sn*-1 acyl chain length can therefore be expressed in the usual form (cf. Marsh, 1991; Seddon et al., 1983):

$$T_t^c = T_t^{c,\infty}[1 - (n_{1,o} - n'_{1,o})/(n_1 - n'_{1,o})] \tag{10}$$

where $T_t^{c,\infty}$ ($\equiv \Delta H'_{inc,1}/\Delta S'_{inc,1}$) is the value of T_t^c extrapolated to infinite *sn*-1 chain length, and $n'_{1,o}$ is defined by the analogue of Eq. 9 for the transition entropy. The fits for the *sn*-2 monounsaturated phosphatidylethanolamines and phosphatidylcholines are shown in Fig. 4 (*top*) and the values of the fitting parameters, $T_t^{c,\infty}$, $(n_{1,o} - n'_{1,o})$, and $n'_{1,o}$ are given in Table 2. These values differ considerably from those for saturated symmetrical diacyl PEs and PCs (Marsh, 1991) because of the presence of the *cis* double bond in the *sn*-2 chain.

The values of the incremental positional enthalpic deficit, h'_c, decrease progressively with *sn*-1 chain length because the normalizing values of ΔH_t^c increase according to Eq. 9 as n_1 increases. Fig. 4 (*bottom*) shows the dependence of h'_c on n_1 for both PE and PC series. This is fitted by the following expression deduced from Eqs. 3 and 9:

$$h'_c = \frac{\Delta h_c/\Delta H'_{inc,1}}{n_1 - n_{1,o}} \tag{11}$$

where the values of the constant fitting parameters, $\Delta h_c/\Delta H'_{inc,1}$ and $n_{1,o}$, are given in Table 2. It is seen that the latter fitted values are reasonably consistent with those deduced from fitting T_t^c in the upper panel of Fig. 4, although the error in $n_{1,o}$ is quite large for the PE series.

In general, therefore, the observed dependence on n_1 is consistent with the model. It should be noted, however, that the n_1 dependence of the fitting parameters is not specified with particularly high precision (see Table 1).

The thermodynamic model as presented above does not differentiate between which of the two segments, the upper Δ-segment or the lower ω-segment, is the longer or shorter. This approximation is justified to the extent that the dependence of transition temperature on *cis* double-bond position is symmetrical about n_c (cf. Fig. 1) and is found to perform reasonably well. To obtain greater precision in their multiple regression analysis, Huang and co-workers (Wang et al., 1994, 1995a,b) fitted the transition temperatures for lipids in which the Δ-segment was the longer separately from those in which the ω-segment was the longer. The present model could be generalized to allow for a residual asymmetry, but this would increase the number of parameters involved.

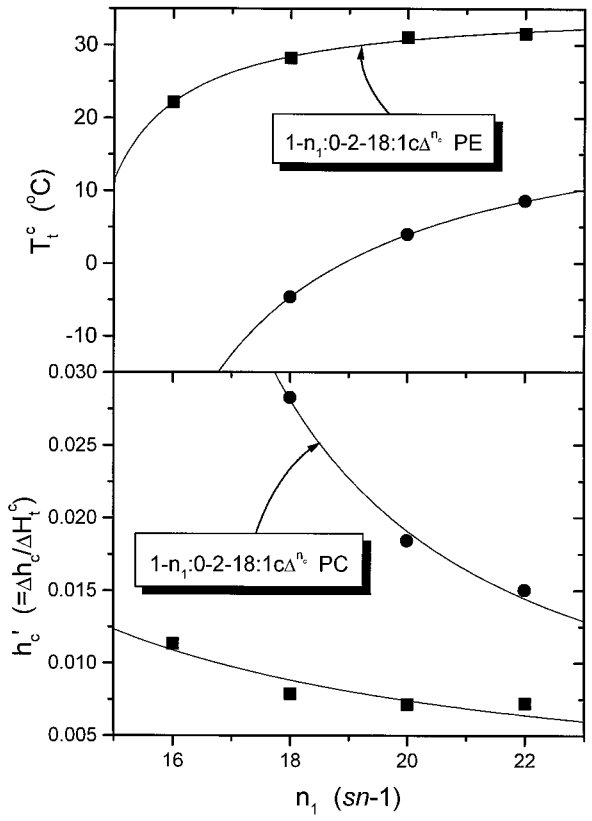


FIGURE 4 Dependence on length, n_1, of the saturated *sn*-1 acyl chain of the parameters fitting the transition temperature as a function of *cis*-bond position in the *sn*-2 chain for 1-(n$_1$:0)-2-(18:1cΔ^{n_u}) phosphatidylethanolamines (■) and phosphatidylcholines (●). (*Top*) Fitted values of the chain-melting transition temperature minimum, T_t^c. (*Bottom*) Fitted values of incremental positional enthalpic deficit, h'_c. The lines are nonlinear least-squares fits of Eqs. 10 and 11, respectively, to the data, with the parameters given in Table 2.

DEPENDENCE ON UNSATURATED CHAIN LENGTH

The most basic prediction of the thermodynamic model is that the critical position, n_c, of the *cis* double bond that corresponds to minimum transition temperature should shift systematically with the length of the unsaturated *sn*-2 chain according to Eq. 7. Fig. 5 gives the dependence of the transition temperature on *cis*-double-bond position for 1-(20:0)-2-(20:1cΔ^{n_u}) phosphatidylcholines and phosphatidylethanolamines. The parameters obtained from fitting Eq. 3 to the data for these longer chain lipids are given in Table 3. Comparison of the values for n_c with those for the corresponding 1-(20:0)-2-(18:1cΔ^{n_u}) phosphatidylcholines and phosphatidylethanolamines in Table 1 reveals the expected increase in n_c with increasing n_2. The increase in n_c by one methylene group for the phosphatidylcholines is quantitatively in agreement with Eq. 7. The increase is somewhat larger for the phosphatidylethanolamines, but in this case the dependence of T_t on n_u is shallower, and therefore n_c is determined less precisely.

TABLE 2 Parameters characterizing the dependence on saturated *sn-1* acyl chain length, n_1, of the predicted minimum chain-melting transition temperature, T_t^c, according to Eq. 10, and of the incremental positional enthalpic deficit, h'_c, according to Eq. 11, for 1-acyl-2-(18:1cΔ^{n_u}) phosphatidylcholines and phosphatidylethanolamines

Lipid	$T_t^{c,\infty}$ (K)	$n_{1,o} - n'_{1,o}$	$n'_{1,o}$	$\Delta h_c/\Delta H'_{inc,1}$	$n_{1,o}$
PC	296.8*	0.438*	13.41*	0.119 ± 0.013	13.8 ± 0.5
PE	308.6 ± 1.7	0.10 ± 0.05	13.7 ± 0.8	0.093 ± 0.028	7.5 ± 3.0

*Not overdetermined.

DEPENDENCE ON LENGTH OF THE LOWER *sn*-2 CHAIN ω-SEGMENT

Fig. 6 gives the dependence of the transition temperatures on the length, n_2, of the monounsaturated *sn*-2 chain for 1-(n_1:0)-2-(n_2:1cΔ^{13}) phosphatidylcholines and phosphatidylethanolamines of fixed *sn*-1 chain length. In these lipid series, the length of the longer, upper Δ-segment of the *sn*-2 chain is kept constant at $n_u = 13$ and the length, $n_2 - n_u$, of the shorter ω-segment below the double bond is varied. For the series with $n_1 = 20$ and 22, the net length of the *sn*-2 chain is always shorter than that of the *sn*-1 chain. Under these circumstances, the dependence of T_t on n_2 is predicted to have the same form as that of T_t^c on n_1 that is given by Eq. 10, but the parameters are, of course, different. From Fig. 6 it is seen that the n_2 dependence of the transition temperature is qualitatively similar for all three lipid series with $n_1 > 18$, although the absolute values of T_t are considerably greater for the phosphatidylethanolamines. However, the transition temperatures increase almost linearly over the range $n_2 = 18$–22, without any pronounced tendency to level off at long chain lengths that is the normal case with fully saturated *sn*-2 chains (see, e.g., Marsh, 1991). The reason for this is partly that the shorter segment of the *sn*-2 chain contributes a reduced incremental enthalpy to the transition (see above). In addition, the mismatch, $n_1 - n_2$, between the *sn*-1 and *sn*-2 chain lengths increases systematically with n_2 for each series in Fig. 6. The enthalpic (and entropic) deficit arising from the nonoverlapping regions of the *sn*-1 and *sn*-2 chains from the same lipid molecule therefore increases with increasing n_2 (see Marsh, 1992). This also retards the tendency of T_t to level off with increasing n_2. Because of the resulting quasilinear n_2 dependence, it is not possible to obtain a unique fit with an expression analogous to Eq. 10. Purely for illustrative purposes, the solid lines in Fig. 6 represent fits with a fixed value of $T_t^\infty = 423$ K, comparable to those found for disaturated phospholipids (Marsh, 1991). Just as for the situation with constant n_2 and variable n_1, the dependence on n_2 with fixed n_1 is calculated with an expression analoguous to Eq. 10 (see Marsh, 1991).

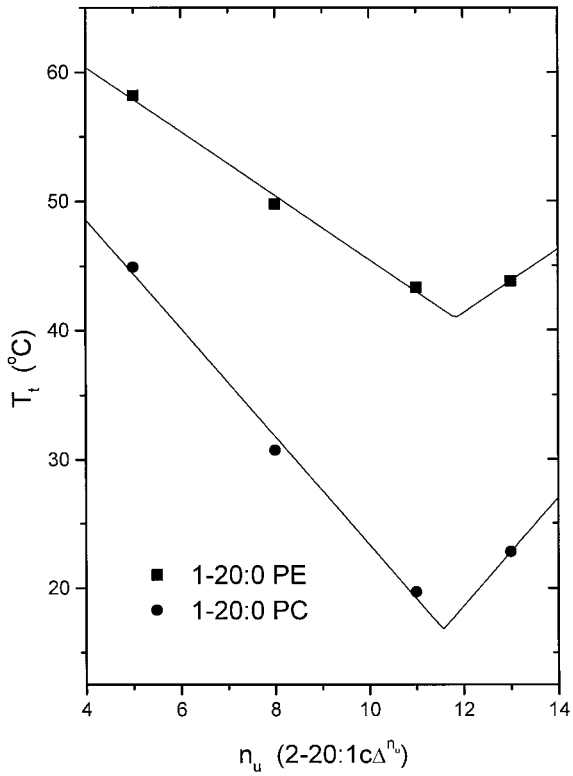


FIGURE 5 Dependence of the chain-melting transition temperature, T_t, of hydrated 1-(20:0)-2-(20:1cΔ^{n_u})-*sn*-glycero-3-phosphocholines (●) and -phosphoethanolamines (■) on the position, n_u, of the *cis*-double bond in the *sn*-2 chain. Data are from Wang et al. (1994, 1995a) and Huang et al. (1996). The solid lines are bilinear fits to Eq. 3 with $s'_c = 0$ and the parameters given in Table 3.

For the 1-(18:0)-2-(n_2:1cΔ^{13}) phosphatidylcholine series, the dependence of the transition temperature on n_2 is qualitatively different from the series with longer *sn*-1 chains (see Fig. 6). This is because the effective length of the *sn*-2 chain, which, as noted above, is given approximately by n_2-2.3 (Wang et al., 1995a), becomes longer than that of the *sn*-1 chain for $n_2 > 20$. At the cross-over point, T_t has its maximum value for lipid series in which the mean chain length is kept constant, because the packing deficit between the *sn*-1 and *sn*-2 chains is reduced to zero (see Huang, 1990; Marsh, 1992). The result of this effect for the 1-(18:0)-2-(n_2:1cΔ^{13}) PCs is that T_t increases much less steeply with increasing n_2 for $n_2 > 20$. For illustrative purposes, a fit using the methods and parameters from analysis of saturated mixed-chain lipids (Marsh, 1992; and see Fig. 7), but with $\Delta n^o = 2.3$ appropriate to a *cis*-monounsaturated *sn*-2 chain, is given in Fig. 6. The characteristic discontinuity in gradient occurs at $n_2 = 20.3$ for this model calculation (see also following section).

It should be noted that for all of the series in Fig. 6, the upper Δ-segment of the *sn*-2 chain is the longer. Taking a mean experimental value of the critical offset $\delta n_c = 1.7$, Eq.

TABLE 3 Parameters characterizing the dependence of the chain-melting transition temperatures of 1-(20:0)-2-(20:1cΔ^{n_u})-*sn*-glycero-3-phosphocholines and -phosphoethanolamines on position, n_u, of the *cis* double bond in the *sn*-2 chain, according to Eq. 3

Lipid	T_t^c (K)	n_c	h_c'	s_c'
PC	289.9 ± 1.1	11.57 ± 0.20	0.0145 ± 0.0011	0.0*
PE	314.1 ± 0.6	11.83 ± 0.21	0.0079 ± 0.006	0.0*

*Fixed.

7 predicts that a critical double-bond position of $n_c = 13$ is achieved for an *sn*-2 chain length of $n_2 = 23.6$. This value is beyond the maximum value of $n_2 = 22$ in Fig. 6.

DEPENDENCE ON LENGTH OF THE UPPER *sn*-2 CHAIN Δ-SEGMENT

Fig. 8 gives the dependence of the transition temperatures on the length, n_2, of the monounsaturated *sn*-2 chain for 1-(n_1:0)-2-(n_2:1cΔ$^{n_2-9}$) phosphatidylcholines and phosphatidylethanolamines of fixed *sn*-1 chain length. In these lipid series, the length of the lower ω-segment of the *sn*-2 chain is kept constant at nine C-atoms, and the length of the Δ-segment above the double bond (i.e., $n_u = n_2$-9) is varied.

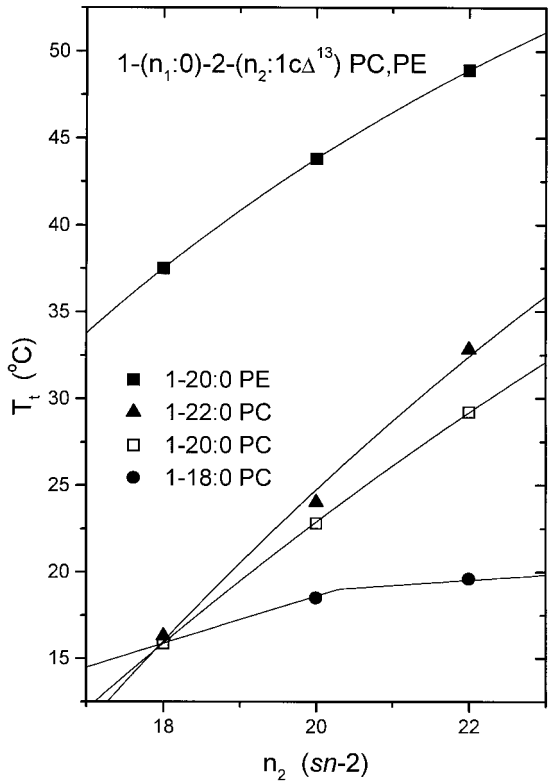
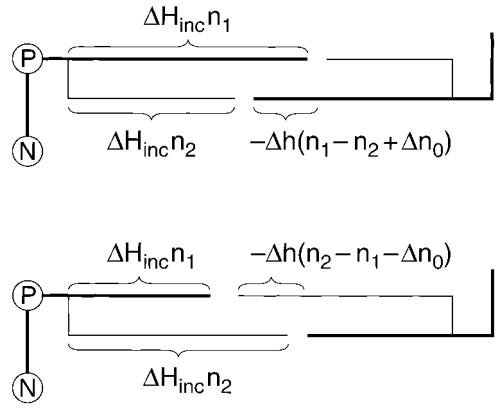


FIGURE 6 Dependence of the chain-melting transition temperature, T_t, of hydrated 1-acyl-2-(n_2:1cΔ^{13})-*sn*-glycero-3-phosphocholines on the length, n_2, of the *sn*-2 chain. The *sn*-1 acyl chain is 18:0 (●), 20:0 (□), or 22:0 (▲). Data are from Wang et al. (1995a,b). Data are also given for 1-(20:0)-2-(n_2:1cΔ^{13})-*sn*-glycero-3-phosphoethanolamines (■) from Wang et al. (1994) and Huang et al. (1996). Solid lines are illustrative fits with certain of the parameters fixed at values obtained from saturated lipids (see text).

This variation has three effects that influence the chain-melting transition temperature. First, increasing n_2 increases the transition temperature by increasing the number of methylene units in the *sn*-2 chain. Second, the critical position, n_c, of the double bond increases with increasing *sn*-2 chain length (see Eq. 7). The distance of the double bond from the position of minimum transition temperature, for a lipid series with constant chain length (i.e., $n_u - n_c = n_2/2$–9-δn_c), consequently also varies. Third, the packing deficit in the region of mismatch between the *sn*-1 and *sn*-2 chains, which is of length $n_1 - n_2 + \Delta n^o$, changes with increasing *sn*-2 chain length (Huang, 1990; Marsh, 1992; and see Fig. 7). Consequently, the *sn*-2 chain length corresponding to exact matching with the *sn*-1 chain ($n_2 = n_1 + \Delta n^o$, which gives the maximum transition temperature for a lipid series with constant mean chain length) increases progressively with the *sn*-1 chain length of the different series. The second and third effects therefore contribute opposite tendencies to the dependence of the transition temperature on *sn*-2 chain length. The relative positions about which the two effects are centered also vary with length of the *sn*-1 chain.



$$\Delta H_t = \Delta H_{inc}(n_1 + n_2) - \Delta h|n_1 - n_2 + \Delta n_0| + \Delta H_0$$

FIGURE 7 Schematic indication of the packing at the chain ends in bilayers of monounsaturated diacyl phospholipids with different *sn*-1 and *sn*-2 chain lengths, n_1 and n_2, respectively. The incremental chain-melting enthalpies per CH_2 group are reduced by an amount Δh for *sn*-1 and *sn*-2 chain sections overlapping from opposed molecules, relative to those from chain sections overlapping in the same diacyl lipid molecule (see Marsh, 1992). The effective length of the *sn*-2 chain, relative to the *sn*-1 chain, is reduced by an amount Δn_o that results from the bend at the C-2 position and the shortening by the *cis* double bond. For simplicity, details of the dependence on the *cis*-bond position (see Fig. 3) are omitted (see text).

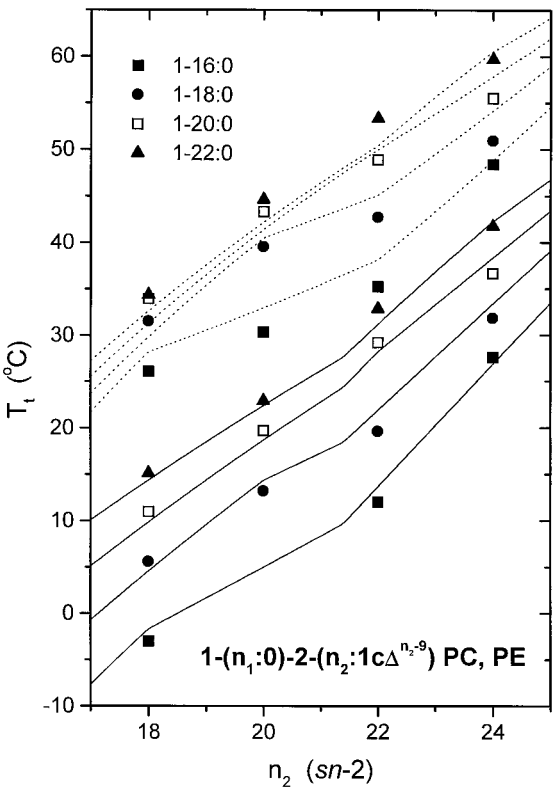


FIGURE 8 Dependence of the chain-melting transition temperature, T_t, of hydrated 1-acyl-2-(n_2:1cΔ^{n_2-9})-*sn*-glycero-3-phosphocholines (PCs) and -phosphoethanolamines (PEs) on the length, n_2, of the *sn*-2 chain. The *sn*-1 acyl chain is 16:0 (■), 18:0 (●), 20:0 (□), or 22:0 (▲). Data are from Wang et al. (1994, 1995a,b) and Huang et al. (1996). Lines for phosphatidylcholines (——) and phosphatidylethanolamines (·····) are predictions from Eq. 13 with a fixed set of parameters for each of the two groups (see text).

The latter two effects mentioned above give rise to the irregular dependence of transition temperature on *sn*-2 chain length that is seen in Fig. 8 for the series with $n_1 = 16$ and 18, particularly in the case of phosphatidylethanolamine. The double bond is situated close to the critical position, n_c, for the lipids with *sn*-2 chain length $n_2 = 22$ for all lipid series in Fig. 8. The *sn*-2 chain length for perfect matching, on the other hand, increases progressively with *sn*-1 chain length. It corresponds approximately to $n_2 \approx 18.3$, 20.3, 22.3, and 24.3 for $n_1 = 16$, 18, 20, and 22, respectively (cf. above). It is this which accounts for the different dependence on n_2 for the series with different values of n_1 in Fig. 8.

To analyze the trends more quantitatively, it is necessary to introduce the *sn*-1/*sn*-2 chain mismatch into Eqs. 6 and 8, as was done previously for fully saturated mixed-chain lipids (Marsh, 1992). The corresponding expression is (see Fig. 7)

$$\Delta H_t^c = \Delta H_{inc}(n_1 + n_2 - n_o) - \Delta h_c n_2/2 - \Delta h|n_1 - n_2 + \Delta n^o| \quad (12)$$

where Δh is the deficit in incremental transition enthalpy arising from the packing mismatch between the *sn*-1 and *sn*-2 chains, and Δn^o is the intrinsic mismatch contributed by the bent conformation at the C-2 position of the *sn*-2 chain and the shortening effect of the *cis* double bond (Huang, 1990; Wang et al., 1995a). An equation with a form similar to that of Eq. 12 applies for the contribution, ΔS_t^c, to the transition entropy in Eq. 2. The general expression for the dependence of the transition temperature on the lengths of the *sn*-1 and *sn*-2 chains and on double-bond position then becomes (from Eqs. 1, 2, 7, 12, and analogs)

$$T_t = T_t^\infty \cdot \frac{n_1 + k_2 n_2 - n_o - h|n_1 - n_2 + \Delta n^o| + h_c|n_u - n_2/2 - \delta n_c|}{n_1 + k_2' n_2 - n_o' - s|n_1 - n_2 + \Delta n^o| + s_c|n_u - n_2/2 - \delta n_c|} \quad (13)$$

where $T_t^\infty = \Delta H_{inc}/\Delta S_{inc}$, $h = \Delta h/\Delta H_{inc}$, $h_c = \Delta h_c/\Delta H_{inc}$, $s = \Delta s/\Delta S_{inc}$, and $s_c = \Delta s_c/\Delta S_{inc}$, and n_o, n_o' characterize end contributions to the transition enthalpy and entropy, respectively, that do not depend on n_1, n_2, or n_u. The reduction factors k_2 and k_2' are introduced to generalize the reduction in enthalpic and entropic contributions, respectively, of the *sn*-2 chain that is given explicitly by $-\Delta h_c n_2/2$ in Eq. 12 for the enthalpy. Specifically they allow for the possibility that even the longer segment of the *sn*-2 chain may contribute less to the transition properties than do the corresponding segments of the *sn*-1 chain.

The approximate values for some of the parameters in Eq. 13 can be estimated from the foregoing analysis and from previous work on saturated mixed-chain phosphatidylcholines (Marsh, 1992). The approximation used so far is that $s_c = 0$, and with this value, the offset of the critical *cis* bond position from the center of the chain is $\delta n_c \approx 1.5$. The effective shortening of the monounsaturated *sn*-2 chain is $\Delta n^o \approx 2.1$, from previous studies on mixed-chain lipids (Marsh, 1992) and allowing for a 0.8 C-atom contribution from the *cis*-double bond (Wang et al., 1995a). Other parameters are difficult to estimate with any degree of certainty because of the normalization involved, i.e., all are quotients of basic parameters. The number of free parameters therefore remains rather large.

Predictions from Eq. 13 using a fixed set of parameters for all of the phosphatidylcholine series (i.e., for all values of n_1) are given by the solid lines in Fig. 8. This parameter set is not unique, but is capable of describing the n_1 and n_2 dependence for all four series with a reasonable degree of precision. The largest deviation of the prediction from the data is 2.3 K. Local minima are evident at $n_2 \approx 21.5$, corresponding to the value of $\delta n_c = 1.7$ used in the predictions (cf. Eq. 7), and the value $\Delta n^o = 2.0$ produces local maxima that increase progressively from $n_2 \approx 18$ for $n_1 = 16$ to $n_2 \approx 24$ for $n_1 = 22$. Predictions using a fixed parameter set for the four phosphatidylethanolamine series are given by the dotted lines in Fig. 8. In this case, the agreement with the experimental data is not so good (maximum deviation 3.2 K). Nonetheless, the fixed values of $\Delta n^o = 2.0$, $\delta n_c = 2.0$ that are used reproduce the qualitative

trend of the n_2 dependences with increasing n_1. It should be noted that a limited dependence of δn_c on n_1 is evident in Table 1, which is not allowed for in Eq. 13. Also, data for $n_2 = 24$ (and $n_u = 15$) were not included in the original analysis of the dependence on double-bond position. Therefore they may not conform exactly with the dependence on n_u that is predicted by Eq. 3. In the following section, it will be seen that there are considerable deviations for $n_u = 15$ when $n_1 = 18$ or 20 (see Li et al., 1998) for phosphatidylethanolamines.

Although it is difficult to read very much into the absolute values of the parameters used for the predictions in Fig. 8, a consistent feature appears to be that reduction factors k_2 and k_2' substantially less than unity are required. This implies that even the longer segment of the *sn*-2 chain contributes less to the calorimetric quantities than does the corresponding section of the *sn*-1 chain.

DOUBLE BONDS CLOSER TO THE CHAIN ENDS

It has been pointed out recently by Li et al. (1998) that extending the phosphatidylethanolamine series given in Fig. 2 for $n_1 = 18$ and 20, to values of $n_u = 6$ or 15, produces an increase in transition temperature that is much greater than that which would be predicted by extrapolation of the bilinear fits that are given in this figure. The implication is that the change in transition enthalpy and/or entropy with double-bond position is considerably larger than that for double bonds positioned closer to the middle of the chain. Fitting the phosphatidylethanolamine transition temperatures solely for positions $n_u = 6$, 7, 13, and 15 (Huang et al., 1996; Li et al., 1998) with Eq. 3 produces parameter values that deviate from those given in Table 1, which are derived for positions closer to the center of the *sn*-2 chain. The predicted values of the minimum transition temperature, $T_t^c = 281 \pm 3$ K and 286 ± 2 K for $n_1 = 18$ and 20, respectively, are ~20° lower than those given in Table 1. The values of the incremental positional enthalpy (obtained with $s_c' = 0$), $h_c' = 0.032 \pm 0.004$ and 0.029 ± 0.002 for $n_1 = 18$ and 20, respectively, are approximately four times larger than those given in Table 1. This means that the shorter segment of the *sn*-2 chain contributes even less to chain melting than would be expected from Table 1 and Eq. 3, which is consistent with the observation that the transition temperatures for $n_u = 6$ or 15 are reasonably close to predictions made assuming that the shorter section of the chain makes a negligible contribution for these particular lipids (Li et al., 1998).

For the di(C18:1cΔ^{n_u}) phosphatidylcholine series, there is direct calorimetric evidence that the transition enthalpy increases more strongly with *cis*-bond position toward the two ends of the chain, in regions up to C-5 and beyond C-12 (Barton and Gunstone, 1975). Treating the outer sections and inner sections separately, the fits of Eq. 3 to the transition temperatures are given in Fig. 9 (*top*). To within the precision of the fits, all yield the same value of n_c, which

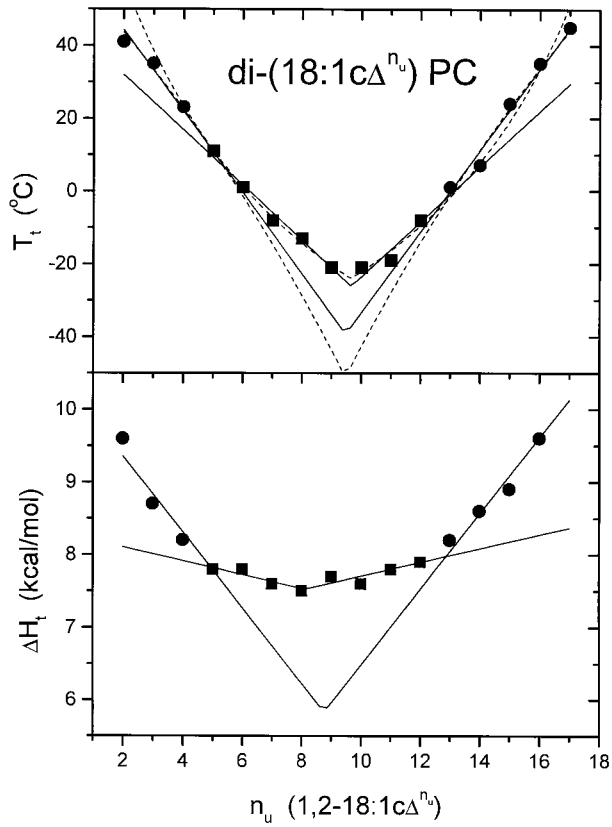


FIGURE 9 Dependence of the chain-melting transition temperature (T_t, *top*) and enthalpy (ΔH_t, *bottom*) of hydrated di(C18:1cΔ^{n_u})-*sn*-glycero-3-phosphocholines on the *cis*-bond position, n_u, in the acyl chains (Barton and Gunstone, 1975). The transition temperatures and enthalpies are fitted by Eqs. 3 and 1, respectively, separately over the regions C2-C4 with C13-C16 (●) and C5-C12 (■). In the upper panel, solid lines are bilinear fits with $s_c' = 0$, and the dashed lines are nonlinear fits with s_c' unconstrained.

agrees with that in Table 1. The value of h_c' is, however, 55% larger, and the interpolated minimum transition temperature, T_t^c, is 13° lower, for the outer sections than for the middle sections of the chain (for the fits with $s_c' = 0$). This is in qualitative agreement with the dependence of the calorimetrically determined enthalpy on *cis*-bond position (Fig. 9, *bottom*). The dependence on n_u is much steeper at the chain ends than at the shallow minimum near the chain center. Quantitatively, the gradients in ΔH_t deduced from the lower part of Fig. 9 correspond to apparent values of h_c' that differ by approximately a factor of 4 from those obtained from bilinear fits to the transition temperature in the upper part of Fig. 9. For the middle part of the chain the calorimetric gradient is less steep, and for the chain ends more steep, than that deduced from the transition temperatures. This could point to differential compensatory entropic contributions, but is most probably due at least in part to the inherently lower sensitivity of the enthalpy measurements to relatively subtle changes that are readily detected in the transition temperature. For instance, the minimum of ΔH_t with respect to n_u is not determined precisely and is appar-

ently shifted with respect to the clear minimum in transition temperature. As already mentioned, the measured transition entropy does not vary systematically to within the experimental precision and therefore is not helpful in resolving this point. The clear result, however, is that the transition enthalpy depends more strongly on double-bond position at the chain ends. Whether this arises from a change in character of the low-temperature phase, e.g., to a more closely packed lamellar crystalline-like phase such as is found on the addition of ethylene glycol to 1-(18:1cΔ^9)-2-(18:1cΔ^9) PC (Lewis et al., 1988), remains to be resolved. An alternative possibility is that the low-temperature state changes to a lamellar gel phase (which putatively would have a considerably lower transition enthalpy for a *cis* double bond close to the chain center). This also is not structurally established but is possibly suggested by the observation of a pretransition for 1-(18:1cΔ^{15})-2-(18:1cΔ^{15}) PC and 1-(18:1cΔ^{16})-2-(18:1cΔ^{16}) PC, where the double bond is close to the chain end (Barton and Gunstone, 1975).

CONCLUSIONS

The thermodynamic model given here fully supports the conclusions reached by Huang and co-workers from a variety of techniques—not least, an impressive body of synthetic and experimental work—on the influence of a single *cis* double bond on the chain melting of unsaturated lipid membranes (Wang et al., 1994, 1995a,b; Huang et al., 1996; Li et al., 1998). The current model does not have the predictive precision of the multiple regression analysis of transition temperatures given by these workers, but rather provides a thermodynamic basis for understanding the dependence on the various structural parameters. It also unifies the treatment of lipids in which the upper Δ-segment of the *sn*-2 chain, above the *cis* double bond, is either longer or shorter than the lower ω-segment. A consistent thermodynamic description is thus obtained for the dependence of transition temperature on both *sn*-1 and *sn*-2 chain lengths over a wide range of variation in the *cis*-double-bond position.

The principal features that emerge from the thermodynamic analysis are the following: 1) The shorter of the *sn*-2 chain segments makes a smaller contribution to the incremental chain-melting enthalpy. It is also possible that it makes a larger contribution to the chain-melting entropy. Even the longer segment of the *sn*-2 chain appears to contribute a smaller incremental enthalpy than does the *sn*-1 chain. 2) The critical *cis*-bond position, n_c, corresponding to the minimum transition temperature at constant chain length, is predicted to be 1.5 CH_2 units beyond the numerical center of the chain. 3) The dependence of transition temperature on the length of the Δ- or ω-segments, with the complementary segment of the *sn*-2 chain kept constant, has two discontinuities. These correspond to the critical *cis*-bond position, n_c, and to the point of exact matching of the *sn*-1 chain length with the effective *sn*-2 chain length. The latter is reduced by $\sim$2–2.3 CH_2 units by the *cis* double bond and the bent configuration at the point of attachment to the glycerol backbone. 4) For double bonds close to either end of the *sn*-2 chain, the incremental positional enthalpy is considerably larger than that for bond positions closer to the chain center.

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