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Rapid report

Chain-melting transition temperatures of phospholipids with acylated or alkylated headgroups (*N*-acyl phosphatidylethanolamines and *O*-alkyl phosphatidic acids), or with α-branched chains

Derek Marsh

Max-Planck-Institut für biophysikalische Chemie, Abteilung Spektroskopie, D-37077 Göttingen, Germany

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Abstract

The biphasic dependence of the chain-melting transition temperature on chainlength, n^N, of the headgroup-attached chain of *N*-acyl phosphatidylethanolamines and of *O*-alkyl phosphatidic acids is interpreted in terms of a different linear dependence of the transition enthalpy and entropy on n^N for long and short chains, respectively. A consistent expression of the form $T_t(n^N) = T_N[|n^N - \Delta n^N| + \Delta h'(n_o^N - n^N)]/[|n^N - \Delta n^N| + \Delta s'(n_o'^N - n^N)]$, where Δn^N is the critical chainlength at which the packing mode of the headgroup-attached chains changes, is found to apply systematically to the transition temperatures for both sets of lipids, over the range $n^N = 2$–18. This thermodynamic analysis demonstrates that the headgroup-attached chain is located in an environment that differs for long and short chains. Similar considerations apply also to phosphatidylcholines with α-branched glycerol-attached chains.

Keywords: *N*-Acyl phosphatidylethanolamine; *O*-Alkyl phosphatidic acid; Chain melting; Transition temperature; Headgroup-attached chain; α-Branched chain; Calorimetry; Bilayer thermodynamics

In addition to the normal diacyl phospholipids that are the principal lipid constituents of biomembranes, it has been found that a further class of phospholipids in which the polar headgroup is acylated by a long fatty acid chain is present as a minority constituent in a wide range of membranes (see e.g. [1]). These unusual lipids are the *N*-acyl phosphatidylethanolamines (*N*-acyl PEs), in which the amine group of phosphoethanolamine is acylated by a long-chain fatty acid. The cellular level of *N*-acyl PEs is increased in response to a variety of membrane stress conditions, and it is thought that the active agent to combat stress is acylethanolamine [2] for which the *N*-acyl PEs form an inert, membrane-compatible reservoir.

Calorimetric studies have shown that the chain-melting transition temperatures, T_t, of *N*-acyl PE bilayer membranes display a systematic biphasic response to the length, n^N, of the *N*-acyl chain when the *sn*-1 and *sn*-2 chains are maintained constant [3]. For shorter chains ($n^N < \Delta n^N$), T_t decreases with increasing n^N, reaches a minimum at $n^N = \Delta n^N$, and then increases for longer chains ($n^N > \Delta n^N$). This is in contrast to the normal dependence on length, n^O, of the phospholipid *O*-acyl glycerol-attached chains, but is a property shared by an analogous systematic series of synthetic lipids, the *O*-alkyl phosphatidic acids in which the phosphate headgroup is alkylated by a saturated hydrocarbon chain [4]. Additionally, a similar dependence of the chain-melting transition temperature is found on the alkyl chainlength of phospholipids with α-branched chains acylated to

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the glycerol backbone [5,6]. Such branched chains are found in the lipids of natural membranes, particularly those of microorganisms. All three series of phospholipids considered here have the common feature that only the modifying chain is varied, over a wide range, and the remainder of the molecule is maintained strictly constant.

Previous work on *N*-acyl phosphatidylethanolamines with long *N*-acyl chains, $n^N=10-18$ [7], has shown that the dependence of the calorimetric properties on length of both *N*-acyl and *O*-acyl (i.e. glycerol) chains can be explained in terms of the mismatch, n^O-n^N, between the two (see Fig. 1). A contribution of this type had already been found to arise from the deficit in chain packing that occurs with mixed-chain diacyl phosphatidylcholines of unequal *sn*-1 and *sn*-2 chainlengths [8]. The basic principles for such an analysis were established by the pioneering work of Huang and coworkers [9,10] on phosphatidylcholines exhibiting partially interdigitated *sn*-1 and *sn*-2 chain packing. In the present paper, the previous thermodynamic treatment is extended to account for the biphasic behaviour of *N*-acyl PEs with shorter headgroup-attached chains. It is found that the dependence of the gel-to-fluid chain-melting transition temperature on n^N can be interpreted consistently for both *N*-acyl phosphatidylethanolamines and *O*-alkyl phosphatidic acids, in terms of the critical chainlength, Δn^N, at which the headgroup chain-packing mode changes between the long and short regimes. In certain respects, this situation has analogies with the change from partially interdigitated to mixed interdigitated *sn*-1/*sn*-2 chain packing that was characterised extensively by Huang [9,10] for mixed chain phosphatidylcholines with increasing chain asymmetry.

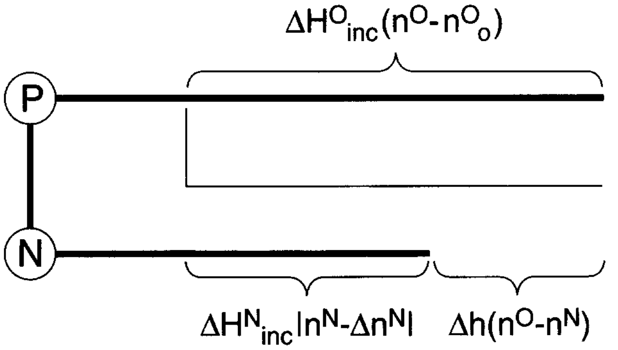

Fig. 1. Schematic diagram of the chain packing in *N*-acyl phosphatidylethanolamines. The basic incremental chain-melting enthalpies (per CH_2 group) are ΔH^O_{inc} for the *O*-acyl chains and ΔH^N_{inc} for the *N*-acyl chain, in the regions where the chains overlap, and Δh is the incremental deficit in the regions where the *O*-acyl and *N*-acyl chains do not overlap (see [7]). The net incremental transition enthalpy is $(\Delta H^O_{inc}+\Delta h)$ for the *O*-acyl chains, $(\Delta H^N_{inc}-\Delta h)$ for long *N*-acyl chains $(n^N>\Delta n^N)$, and $-(\Delta H^N_{inc}+\Delta h)$ for short *N*-acyl chains $(n^N<\Delta n^N)$. The end contribution to the transition enthalpy is $-(\Delta H^O_{inc}n^O_o+\Delta H^N_{inc}\Delta n^N)$ for long *N*-acyl chains $(n^N>\Delta n^N)$, and for short *N*-acyl chains $(n^N<\Delta n^N)$, it is $-(\Delta H^O_{inc}n^O_o-\Delta H^N_{inc}\Delta n^N)$.

Fig. 2 gives the chain-melting transition temperatures as a function of systematically increasing *N*-acyl chainlength for two series of phosphatidylethanolamines of fixed *O*-acyl chain composition. These series are *N*-acyl dipalmitoyl phosphatidylethanolamines (1,2-dipalmitoyl-*sn*-glycero-3-phospho-*O*-(*N*-acyl)ethanolamine; *N*-acyl DPPE) which have C(16:0) *O*-acyl chains, and *N*-acylated phosphatidylethanolamines that are formed by headgroup transphosphatidylation from egg yolk phosphatidylcholine (*N*-acyl TPE). The latter have a fixed *O*-acyl chain composition that consists primarily of *sn*-1 palmitoyl and *sn*-2 oleoyl chains. Also included in Fig. 2 are the transition temperatures of dipalmitoyl phosphatidic acids (1,2-dipalmitoyl-*sn*-glycero-3-(*O*-alkyl)phosphoric acid; *O*-alkyl DPPA) with *O*-alkyl chains of systematically increasing length attached to the headgroup phosphate. The transition temperatures of the *O*-alkyl DPPAs lie close to the corresponding ones for the *N*-acyl DPPEs, probably because these have an identical *O*-acyl glycerol chain composition.

The biphasic dependence on *N*-acyl chainlength evident in Fig. 2 indicates that, for *N*-acyl chains shorter than a critical length, Δn^N, the packing of the *N*-acyl chains changes from that which is characteristic of the longer-chain *N*-acyl phosphatidylethanolamines analysed previously [7]. The most likely candidate for this change is that the *N*-acyl chain becomes no longer buried in the hydrophobic interior of the membrane. Not surprisingly, the critical length for the change in the *N*-acyl chain packing mode depends on the fixed *O*-acyl chain composition. For *N*-acyl DPPEs and *O*-alkyl DPPAs, the critical lengths of the headgroup-attached chain are similar: $\Delta n^N \sim 9$, whereas that for *N*-acyl TPEs is smaller: $\Delta n^N \sim 7$.

To take specific account of the chain packing def-

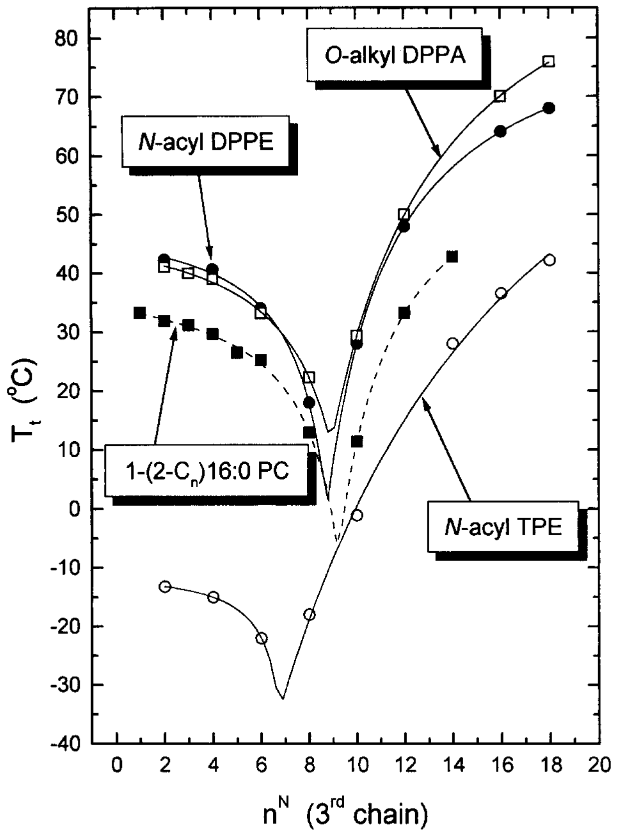

Fig. 2. Dependence of chain-melting transition temperature on *N*-acyl chainlength, n^N, for *N*-acyl dipalmitoyl phosphatidylethanolamines (●, *N*-acyl DPPE) and for *N*-acyl transphosphatidylated phosphatidylethanolamines (○, *N*-acyl TPE); that for *O*-alkyl dipalmitoyl phosphatidic acids (□, *O*-alkyl DPPA) on *O*-alkyl chainlength; and that for 1-(2-alkyl)palmitoyl-2-hexadecyl phosphatidylcholines (■, 1-(2-C$_n$)16:0 PC) on α-branched alkyl chainlength. Data for *N*-acyl TPE are from [3], and those for *N*-acyl DPPE are additionally from [11] ($n^N = 6$, 18), [12] ($n^N = 8$, 10) and [7] ($n^N = 16$). Data for *O*-alkyl DPPA are from [4]. Data for 1-(2-C$_n$)16:0 PC are from [6]. Lines are non-linear least squares fits to Eq. 5. The fitting parameters are given in Table 1.

icit in the region of mismatch between the *N*-acyl and *O*-acyl chains, the transition enthalpy and entropy for long *N*-acyl chains were expressed previously as [7]:

$$\Delta H_t = \Delta H^O_{inc}\,(n^O - n^O_o) + \Delta H^N_{inc}\cdot n^N + \Delta h(n^O - n^N) \qquad (1)$$

$$\Delta S_t = \Delta S^O_{inc}\,(n^O - n^{O\prime}_o) + \Delta S^N_{inc}\cdot n^N + \Delta s(n^O - n^N) \qquad (2)$$

where ΔH^O_{inc}, ΔS^O_{inc} are the incremental transition enthalpy and entropy, respectively, for the *O*-acyl chains and ΔH^N_{inc}, ΔS^N_{inc} are the corresponding values for the *N*-acyl chain. The end contributions to the transition enthalpy and transition entropy are $\Delta H^O_{inc} n^O_o$ and $\Delta S^O_{inc} n^{O\prime}_o$, respectively, for the *O*-acyl chains and those for the *N*-acyl chains are given by the final terms on the right-hand side of Eqs. 1 and 2 (see Fig. 1). These latter terms correspond to effective packing deficits in the region of *O*-acyl and *N*-acyl chain mismatch. In their most general form, these would contain an additional term that represents an end contribution to the mismatch. A somewhat simplified description that concentrates solely on the chainlength dependence for *N*-acyl chains of general length is now given below. The complete derivation is given in the Appendix.

For the case of both short and long headgroup-attached chains, a change in *N*-acyl chain packing mode that will affect all thermodynamic parameters of the chain-melting transition takes place at the critical chainlength Δn^N. To a first approximation, a biphasic linear dependence on n^N may be assumed for both the transition enthalpy and transition entropy when n^O is maintained constant (cf. Eqs. 1 and 2, and see the Appendix):

$$\Delta H_t(n^N) = \Delta H^N_{inc}\,|n^N - \Delta n^N| + \Delta h(n^N_O - n^N) \qquad (3)$$

$$\Delta S_t(n^N) = \Delta S^N_{inc}\,|n^N - \Delta n^N| + \Delta s(n_O{}'^N - n^N) \qquad (4)$$

where the switching function at $n^N = \Delta n^N$ is similar to that used previously in the case of *sn*-1/*sn*-2 chain asymmetry for mixed-chain diacyl phosphatidylcholines [8]. The dependence on length of the headgroup-attached chain that is given by Eq. 3 has different gradients and intercepts in the two regimes, but a common value of the transition enthalpy $\Delta H_t(\Delta n^N) = \Delta h(n^N_O - \Delta n^N)$ at the critical *N*-acyl chainlength (and correspondingly for the transition entropy from Eq. 4). For $n^N > \Delta n^N$, the incremental transition enthalpy per *N*-acyl CH$_2$ group is $+(\Delta H^N_{inc} - \Delta h)$, and for $n^N < \Delta n^N$ it is $-(\Delta H^N_{inc} + \Delta h)$, i.e. of different magnitude and opposite sign. The predictions of Eqs. 3 and 4 are borne out, at least qualitatively, by the experimentally measured enthalpies for a limited series of *N*-acyl DPPEs (see [3,7]). From Eqs. 3 and 4, the resulting dependence of the chain-melting transition temperature ($T_t = \Delta H_t / \Delta S_t$) on *N*-acyl chainlength for a fixed value of n^O is predicted to be:

$$T_t(n^N) = T_N \cdot \frac{|n^N - \Delta n^N| + \Delta h'(n_O^N - n^N)}{|n^N - \Delta n^N| + \Delta s'(n_O{}'^N - n^N)} \tag{5}$$

where T_N ($= \Delta H_{\mathrm{inc}}^N / \Delta S_{\mathrm{inc}}^N$) and $\Delta h'$ ($= \Delta h / \Delta H_{\mathrm{inc}}^N$), $\Delta s'$ ($= \Delta s / \Delta S_{\mathrm{inc}}^N$) are fitting parameters that are related directly to the *N*-acyl chainlength dependence of the calorimetric enthalpy and entropy.

Non-linear least-squares fits of Eq. 5 to the experimental data are given by the continuous lines in Fig. 2, for each series of lipids with systematically varied length of the headgroup-attached chain and fixed composition of the glycerol-attached chains. Corresponding values of the fitting parameters are given in Table 1. From the quality of the fits in Fig. 2, it is seen that Eq. 5 is capable of describing the dependence on *N*-acyl chainlength of the transition temperatures of both *N*-acyl DPPEs and *N*-acyl TPEs with a high degree of accuracy. The same also holds true for the dependence of the *O*-alkyl DPPA transition temperatures on headgroup alkyl chainlength, over a wide range that encompasses both the long and short chainlength regimes. This lends considerable support to the thermodynamic model used in deriving Eq. 5, and further emphasises the change in packing mode of the headgroup-attached chains that occurs at the critical chainlength. The fitting parameters (see Table 1) indicate that *N*-acyl chains with $n^N < \Delta n^N$ proportionately make a considerably smaller (i.e. negative) contribution to the total calorimetric enthalpy and entropy than do those with $n^N > \Delta n^N$, because the signs of $\Delta h'$ and $\Delta s'$ are both positive (cf. above). Presumably, the *N*-acyl chains with $n^N < \Delta n^N$ are less well packed than are those with $n^N > \Delta n^N$, which pack together with the *O*-acyl chains in the hydrophobic core of the membrane. This fact is also reflected in the asymmetry of the chainlength dependence of the transition temperatures that is seen in Fig. 2.

Thermodynamic analysis of the dependence of the chain-melting transition temperatures on length of the headgroup-attached chains therefore supports the assignment given above of the environment of chains shorter than the critical length, for two different lipid classes of this type. The shorter chains are located most likely on the aqueous side of the polar–apolar interface. This can be deduced from the continuity in thermodynamic behaviour with those lipids whose chains are simply too short to dip back into the membrane. Chains longer than the critical length, on the other hand, are buried in the hydrocarbon region of the membrane. Further evidence for the latter assignment comes from the fact that, in favourable circumstances, long *N*-acyl chains are capable of inducing inverted hexagonal phases [11,13,14]. The methods of analysis used here are therefore helpful for identifying relative locations of headgroup-attached phospholipid chains. The analysis can be extended to accommodate the general dependence of the transition temperature on length of the chains attached to the glycerol backbone, as well as of those attached to the lipid headgroup. To aid future work, these results for the general case are given in the Appendix.

Finally, attention is drawn to a rather different series of phospholipids which nevertheless exhibits a rather similar thermotropic behaviour to that of the phospholipids with headgroup-attached chains. Fig. 2 gives the dependence of the chain-melting transition temperatures of 1-(2-alkyl)palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholines (1-(2-alkyl) PC) on the length of the α-alkyl branch in

Table 1
Values of parameters characterizing the dependence of the chain-melting transition temperatures of *N*-acyl phosphatidylethanolamines and *O*-alkyl phosphatidic acids on length of the headgroup-attached chains

Lipid	T_N (K)	$\Delta h'$	$\Delta s'$	n_o^N	$n_o{}'^N$	Δn^N
N-acyl DPPE	336.9 ± 3.2	0.28 ± 0.10	0.33 ± 0.10	14.0 ± 2.6	14.3 ± 2.5	8.73 ± 0.09
N-acyl TPE	270 ± 24	0.84 ± 0.32	0.89 ± 0.22	8.0 ± 4.4	8.1 ± 4.6	6.7 ± 1.0
O-alkyl DPPA	344.0 ± 3.3	0.260 ± 0.077	0.335 ± 0.076	18.8 ± 3.9	18.3 ± 3.2	8.91 ± 0.06
1-(2-alkyl) PC	325.4 ± 2.6	0.13 ± 0.13	0.17 ± 0.14	21.7 ± 13.5	21.0 ± 10.3	9.2 ± 0.1

Parameters for the dependence on α-alkyl chainlength of α-branched phosphatidylcholines are also given. The chainlength dependences given in Fig. 2 are fitted with Eq. 5.

the *sn*-1 chain. Again, the dependence is biphasic when all other parts of the lipid structure are maintained constant, with a minimum in transition temperature occurring at a critical chainlength of the α-branch of $\Delta n^N \sim 9$. This value is similar to that for the headgroup-attached chain of the *N*-acyl DPPEs and *O*-alkyl DPPAs, which also have two C-16 length chains attached to the glycerol backbone. The fit of Eq. 5 to the transition temperatures of the α-branched phosphatidylcholines (PC) is shown in Fig. 2, with the fitting parameters given in Table 1. Again, the same formulation is capable of describing the chainlength dependence, in this case for the α-branch of the *sn*-1 chain, although not all fitting parameters are determined with the same precision as for the *N*-acyl PEs and *O*-alkyl DPPAs. It was suggested previously that short α-alkyl chains of the α-branched PCs might tend toward the polar headgroup region and stabilise lamellar phases, whereas longer α-alkyl chains must be located in the hydrophobic region because they are able to induce inverted hexagonal phases [5]. This situation, although very possible, is not so clear-cut as in the case of the headgroup-attached chains. The long and short α-alkyl chain analogues form different both gel and fluid phases and this alone may be sufficient to account for the biphasic behaviour of the transition temperature. Symmetrical diacyl phosphatidylcholines with α-branched chains also display a behaviour qualitatively similar to that shown for the *sn*-1 α-branched analogues in Fig. 2 [5]. However, the limited number of data points and rather complex phase behaviour [5,15] does not currently permit an unambiguous analysis for this latter case.

I thank Prof. Alfred Blume for information regarding the calorimetric properties of *O*-alkyl PAs.

Appendix

The global dependence of transition temperature on both *N*-acyl and *O*-acyl chainlengths of glycerolipids is given here. This general treatment, which includes the chains attached to the glycerol backbone, is more complicated than that necessary for interpreting the present data. It is presented for completeness, and also to establish the relationship with the previous analysis of *O*-acyl and *N*-acyl chainlength dependence [7], which was restricted to long *N*-acyl chains. The generalisation of Eqs. 1 and 2 to take account of the change in *N*-acyl chain packing mode at the critical chainlength Δn^N is the following (see Fig. 1):

$$\Delta H_t = \Delta H^O_{\text{inc}}(n^O - n^O_o) + \Delta H^N_{\text{inc}}|n^N - \Delta n^N| + \Delta h(n^O - n^N) \qquad \text{A.1}$$

$$\Delta S_t = \Delta S^O_{\text{inc}}(n^O - n^{O\prime}_o) + \Delta S^N_{\text{inc}}|n^N - \Delta n^N| + \Delta s(n^O - n^N) \qquad \text{A.2}$$

For constant *O*-acyl chainlength, these expressions reduce to Eqs. 3 and 4, where the compound end contributions ($\Delta h \cdot n^N_o$ and $\Delta s \cdot n'^N_o$) in the latter are given, respectively, by:

$$n^N_o = \frac{\Delta H^O_{\text{inc}}}{\Delta h}\left[\left(1 + \frac{\Delta h}{\Delta H^O_{\text{inc}}}\right)n^O - n^O_o\right] \qquad \text{A.3}$$

$$n_o{}'^N = \frac{\Delta S^O_{\text{inc}}}{\Delta S}\left[\left(1 + \frac{\Delta s}{\Delta S^O_{\text{inc}}}\right)n^O - n^{O\prime}_o\right] \qquad \text{A.4}$$

The general expression for the dependence of the transition temperature on n^O and n^N is then from Eqs. A1 and A2 (cf. Eq. 5):

$$T_t(n^N, n^O) = T_N \cdot \frac{|n^N - \Delta n^N| + \Delta h'(n^O - n^N) + \Delta h^O(n^O - n^O_o)}{|n^N - \Delta n^N| + \Delta s'(n^O - n^N) + \Delta s^O(n^O - n^{O\prime}_o)} \qquad \text{A.5}$$

where $\Delta h^O = \Delta H^O_{\text{inc}}/\Delta H^N_{\text{inc}}$ and $\Delta s^O = \Delta S^O_{\text{inc}}/\Delta S^N_{\text{inc}}$, and all other constants are as previously defined. An alternative and completely equivalent expression is:

$$T_t(n^O, n^N) = T_O \cdot \frac{n^O - n^O_o + \Delta h^N[|n^N - \Delta n^N| + \Delta h'(n^O - n^N)]}{n^O - n^{O\prime}_o + \Delta s^N[|n^N - \Delta n^N| + \Delta s'(n^O - n^N)]} \qquad \text{A.6}$$

where $T_O = \Delta H^O_{\text{inc}}/\Delta S^O_{\text{inc}}$, $\Delta h^N = \Delta H^N_{\text{inc}}/\Delta H^O_{\text{inc}}$ and $\Delta s^N = \Delta S^N_{\text{inc}}/\Delta S^O_{\text{inc}}$. Eq. A5 places the emphasis on the headgroup-attached chains, and Eq. A6 correspondingly places the emphasis on the chains attached to the glycerol backbone.

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