Differential Scanning Calorimetry of Thermotropic Phase Transitions in Vitaminylated Lipids: Aqueous Dispersions of N-Biotinyl Phosphatidylethanolamines

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ABSTRACT The thermotropic phase behavior of a homologous series of saturated diacyl phosphatidylethanolamines in which the headgroup is N-derivatized with biotin has been investigated by differential scanning calorimetry. In 1 M NaCl, derivatives with acyl chainlengths from C(12:0) to C(20:0) all exhibit sharp chain-melting phase transitions, which are reversible with a hysteresis of 1.5° or less, except for the C(12:0) lipid which has a transition temperature below 0°C. The transition enthalpy and the transition entropy depend approximately linearly on the lipid chainlength, with incremental values per CH₂ group that are very similar to those observed for the corresponding underivatized phosphatidylethanolamines in aqueous dispersion. The chainlength-independent contribution to the transition enthalpy is significantly smaller than that for the underivatized phosphatidylethanolamines, and that for the transition entropy is much smaller, the latter suggesting that the N-biotinylated phosphatidylethanolamine headgroups are differently hydrated from those of the underivatized lipids. The gel-to-fluid phase transition temperatures of the N-vitaminylated lipids are lower than those of the parent phosphatidylethanolamines, and their chainlength dependence conforms with that predicted by assuming that the transition enthalpy and entropy are linearly dependent on chainlength. Although the chain-melting phase behavior is generally similar to that of the parent phosphatidylethanolamines, the gel phases (and the fluid phases in the case of chainlengths C(12:0) to C(16:0)) have a different lyotropic structure in the two cases, and this is reflected in the chainlength-independent contributions to the thermodynamic parameters. In the absence of salt, the thermotropic phase behavior of aqueous dispersions of the N-biotinyl phosphatidylethanolamines is considerably more complex. The transition temperatures are consistently lower than those in 1 M NaCl, but the transitions are broader, contain multiple peaks and exhibit a much larger hysteresis between heating and cooling scans. Additionally, the lipids with shorter chainlengths exhibit metastability in the absence of salt, converting from a micellar solution to a lamellar gel phase only after incubation at low temperature with freeze-thaw cycling.

INTRODUCTION

Phospholipids with derivatized headgroups can be used for attaching further molecules either for targeting of liposomes or constructing molecular devices from monolayer lipid arrays (see, e.g., Urdal and Hakomori (1980), Loughrey et al. (1987), and Ahlers et al. (1989)). Of the possible derivatives, phosphatidylethanolamines to which the vitamin biotin is attached (Bayer et al., 1979; Blankenburg et al., 1989) are especially useful because of the extremely high affinity of avidin for biotin (Green, 1975). Not only are lipid systems with incorporated vitamins of intrinsic biological interest, but also biotinylated phosphatidylethanolamine (biotin-PE) is of use for ultrastructural localization in biological systems (Bayer et al., 1979), or for the formation of two-dimensional arrays for protein structure determination by electron crystallography (Blankenburg et al., 1989; Darst et al., 1991).

The biotin-PEs are interesting also from the point of view of phospholipid model membranes, because the stable phases of their aqueous dispersions differ in many instances from those normally encountered with the common diacyl phospholipid species (Swamy et al., 1993). They form interdigitated lamellar gel phases (Lₐ) and, for the shorter chainlengths (C(12:0) and C(14:0)) in the presence of salt, they form novel nonlamellar fluid phases of an isotropic type (Iₘₐₜ) that consist of aggregated normal micelles. Whereas in the presence of salt the fluid phases for the biotin-PEs of longer chainlengths (C(18:0) and C(20:0)) are lamellar (Lₐ), in the absence of salt the fluid phases are micellar (Mₐ) for all chainlengths. For the derivative of intermediate chainlength, C(16:0), in the presence of salt, the Iₘₐₜ phase is formed immediately on chain melting, but then a transition takes place to the lamellar Lₐ phase at higher temperature within the fluid phase.

From the point of view of the membrane interactions of biotin-PEs, the thermotropic phase behavior of these unusual phospholipids is of particular relevance (cf. Lewis and McElhaney, 1992). In the present work we have studied the chain-melting transitions in aqueous dispersions of saturated diacyl biotin-PEs with chainlengths from C(12:0) to C(20:0), in the presence and in the absence of salt, by differential scanning calorimetry.
calorimetry. The calorimetric properties of the biotin-PEs are compared with those of dispersions of the parent underivatized phosphatidylethanolamines in order to gain information on the interactions between the biotin headgroups in the aqueous aggregates. The biotin-PEs are negatively charged at neutral pH, and therefore the properties at high ionic strength (1 M NaCl) for which the surface electrostatics are fully shielded (Cevc et al., 1980; 1981) can be compared directly with those of the zwitterionic phosphatidylethanolamines, whereas the behavior at low ionic strength (no salt) evidences the additional effects of the charges on the phospholipid headgroups.

EXPERIMENTAL PROCEDURES

Materials

Saturated diacyl N-biotinylated phosphatidylethanolamines with symmetrical chains of lengths from 12 to 20 C-atoms (DLBPE, DMBPE, DPBPE, DSBPE, and DABPE (see Fig. 1)) were synthesized from the corresponding underivatized phosphatidylethanolamines (from Avanti, Birmingham, AL, and Fluka, Buchs, Switzerland) and biotinyl-N-hydroxysuccinimide (from Sigma Chemical Co., St. Louis, MO) according to Bayer and Wilchek (1974) and Bayer et al. (1979). Spin-labeled biotin-PE bearing the nitroxide oxazoline ring on the 5 C-atom of the sn-2 chain (5-BPESL) was synthesized in an analogous manner from the corresponding spin-labeled phosphatidylethanolamine which was prepared as described by Marsh and Watts (1982).

Sample preparation

Samples for differential scanning calorimetry were prepared by weighing a known amount (2-5 mg) of the required biotin-PE into the DSC sample ampoule and then adding 0.5 ml of the required buffer solution. The ampoule was then sealed with the screw-top lid. Hydration was achieved by heating the sample above the thermotropic phase transition in the calorimeter. The reference ampoule either contained an equal volume of the buffer alone or was empty, and a sample containing an equal volume of the buffer alone was used to subtract the baseline. Samples for ESR spectroscopy were prepared by dissolving 1 mg of biotin-PE with 1 mole% of 5-BPESL spin label in dichloromethane. The solvent was evaporated with a stream of nitrogen and the residual traces of solvent removed under vacuum overnight. The dry lipid was then hydrated with 100 µl of the required buffer by warming above the phase transition and vortexing. The dispersion was then transferred to a 100-µl capillary and centrifuged at 10,000 rpm in a Biofuge to obtain a tightly packed lipid pellet. Excess supernatant was then removed and the capillary was flame sealed.

Differential scanning calorimetry

Differential scanning calorimetry was performed with a Model 4207 heat-flow calorimeter from Hart Scientific. Samples consisted of 2 mg of lipid dispersed in 0.5 ml of buffer, and scan rates were either 4° or 9°/h. The buffer used was 10 mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (Hepes), 1 mM EDTA, pH 7.4, with or without 1 M NaCl. Equivalent results were obtained either with buffer in the reference cell or by making a baseline correction with a blank buffer sample. Transition enthalpies were evaluated by using the peak integration software supplied with the instrument and using the measured value of the scan rate at the point of the transition.

Electron spin resonance spectroscopy

ESR spectra were recorded on a Varian E-12 Century Line 9-GHz spectrometer equipped with nitrogen gas flow temperature regulation. Samples were contained in sealed 1-mm ID glass capillaries accommodated within a standard quartz ESR tube which contained silicone oil for thermal stability. Temperature was measured with a thermocouple situated in the silicone oil just above the top of the ESR cavity.

RESULTS

The thermotropic phase transitions of saturated, symmetrical, diacyl biotinyl phosphatidylethanolamines of even chainlengths from C(12:0) to C(20:0), dispersed in neutral buffer either without additional salt or with 1 M NaCl, have been investigated by high-sensitivity differential scanning calorimetry at relatively low scan rates. At 1 M NaCl, the electrostatic interactions of these charged lipids will be screened almost totally (Cevc et al., 1980), but binding of salt is unlikely to be appreciable (Cevc et al., 1981).

Calorimetry of N-biotinyl phosphatidylethanolamines in 1 M NaCl

The differential scanning calorigrams of saturated diacyl N-biotinyl phosphatidylethanolamines of chainlengths from C(12:0) to C(20:0) dispersed in 1 M NaCl, 10 mM Hepes, 1 mM EDTA, pH 7.4, are given in Fig. 2. With the exception of DLBPE, the lipid dispersions all give sharp, reversible thermotropic transitions with a hysteresis of 1-1.5° between heating and cooling scans for scan rates of 4°/h. The half-widths of the transitions are in the range of 0.3-0.4°, with the
FIGURE 2 Differential scanning calorigrams of saturated diacyl N-biotinyl phosphatidylethanolamines (4 mg/ml) dispersed in 1 M NaCl, 10 mM Hepes, 1 mM EDTA, pH 7.4. Heating (endothermic transitions) and cooling (exothermic transitions) scans are shown, with heating and cooling rates of 4°/h, except for DLBPE where the cooling rate is < 4°/h. (a) DLBPE, C(12:0) chains; (b) DMBPE, C(14:0) chains; (c) DPBPE, C(16:0) chains; (d) DSBPE, C(18:0) chains; and (e) DABPE, C(20:0) chains.
sharpest transition being for DMBPE. The transitions on heating are endothermic (upward peaks) and on cooling are exothermic (downward peaks). For DLBPE, the transition takes place at below 0°C, and this gives rise to complications in the heating scans. On cooling, a single sharp transition is obtained for DLBPE, and this probably corresponds to optimum supercooling of the dispersing buffer solution. No other thermotropic transitions than those shown were detected over the range ~20°C to +80°C for DLBPE, 2°C to 60°C for DMBPE, 5°C to 85°C for DPBPE, 10°C to 70°C for DSBPE, and 20°C to 90°C for DABPE. In particular, no calorimetric endo-/exotherm was detected corresponding to the structural transition from the $I_m$ to the $L_\alpha$ phase that takes place with DPBPE dispersions in 1 M NaCl over the temperature range from 60°C to 70°C (Swamy et al., 1993).

The transition temperatures, transition enthalpies, and transition entropies obtained from heating scans for the biotin-PEs of different chainlengths in 1 M NaCl, 10 mM Hepes, 1 mM EDTA, pH 7.4, are given in Table 1. The values for the transition entropy were calculated assuming a first-order transition. With the exception of DLBPE, the values obtained for the transition enthalpy from both heating and cooling scans were rather similar (0.0–0.5 kcal/mol difference). For the former, data is given for the cooling scan (cf. above). The data were obtained at a lipid concentration of 4 mg/ml and a scan rate of 4°C/h, except for DLBPE where the cooling rate was even slower. Transition enthalpies obtained at a scan rate of 9°C/h were essentially identical to those obtained at a scan rate of 4°C/h, and showed no dependence on lipid concentration over the range 3 to 10 mg/ml.

**Table 1** Transition temperatures, $T_n$, transition enthalpies, $\Delta H_n$, and transition entropies, $\Delta S_n$, of N-biotinyl phosphatidylethanolamines of different chainlengths, $n$, dispersed in 10 mM Hepes, 1 mM EDTA, pH 7.4, with or without 1 M NaCl.

<table>
<thead>
<tr>
<th>$n$</th>
<th>$T_n$ °C</th>
<th>$\Delta H_n$ kcal/mol</th>
<th>$\Delta S_n$ cal/mol/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-atom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 M NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12*</td>
<td>-9.5</td>
<td>4.5 ± 0.2</td>
<td>17.1 ± 0.7</td>
</tr>
<tr>
<td>14</td>
<td>18.3</td>
<td>8.1 ± 0.1</td>
<td>27.9 ± 0.2</td>
</tr>
<tr>
<td>16</td>
<td>40.7</td>
<td>10.0 ± 0.3</td>
<td>31.9 ± 0.9</td>
</tr>
<tr>
<td>18</td>
<td>55.0</td>
<td>12.5 ± 0.3</td>
<td>38.1 ± 1.0</td>
</tr>
<tr>
<td>20</td>
<td>65.5</td>
<td>14.3 ± 0.3</td>
<td>42.2 ± 1.0</td>
</tr>
<tr>
<td>0 M NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>7.8</td>
<td>8.0 ± 0.5</td>
<td>28.6 ± 1.0</td>
</tr>
<tr>
<td>16</td>
<td>29</td>
<td>10.4 ± 0.1</td>
<td>34.3 ± 0.4</td>
</tr>
<tr>
<td>18</td>
<td>45.5</td>
<td>12.8 ± 0.2</td>
<td>40.3 ± 0.5</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>14.4 ± 0.4</td>
<td>43.2 ± 1.2</td>
</tr>
</tbody>
</table>

* Determined from cooling scans. All other data are from heating scans.

The overall sizes of the transition enthalpies given in Table 1 suggest that the thermotropic transition observed corresponds to a chain-melting transition from an ordered gel to a fluid liquid-crystalline phase (cf. Marsh, 1990). This identification of the transition is confirmed by electron spin resonance measurements of the changes in the lipid chain dynamics for a spin-labeled biotin-PE used as a probe in the lipid dispersions. The temperature dependence of the ESR spectra of the 5-BPESL spin label, which bears a nitroxide radical on the 5 C-atom of the sn-2 chain, in dispersions of DMBPE in 1 M NaCl, 10 mM Hepes, 0.1 mM EDTA, pH 7.4, is given in Fig. 4. At low temperatures the ESR spectra are essentially immobilized powder patterns with the full hyperfine anisotropy (outer hyperfine splitting of 65 G), characteristic of a phospholipid gel phase. The ESR spectra give no indication of any spin-spin broadening which might arise from exclusion of the spin label from the bulk lipid, hence indicating that the lipid chain immobilization corresponds to a gel phase rather than to a tightly packed crystalline phospholipid phase. The ESR spectra change relatively little with increasing temperature up to 17°C, confirming that the lipid remains in a gel phase over this temperature range. At 18°C the ESR spectra change abruptly from a powder pattern to an axial lineshape with smaller hyperfine anisotropy, which is characteristic of a fluid liquid-crystalline phase (see, e.g., Marsh and Watts (1981)). The phase transition is evidenced in the ESR spectra by the sharp decrease in the outer hyperfine splitting from 63 G at 17°C to 55.5 G at 19°C. The outer hyperfine splitting then decreases steadily with increasing temperature, to a value of 50.5 G at 34°C, as expected also for a fluid liquid-crystalline phase. Similar conclusions with respect to the chain-melting nature of the phase transition are reached from the spin label
ESR spectra of biotin-PEs of other chainlengths and also of biotin-PEs in the absence of salt (data not shown).

DISCUSSION

Aqueous dispersions of the biotin-PEs of different chainlengths undergo thermotropic transitions which correspond to melting of the lipid chains from a gel phase to a fluid liquid crystalline phase. Only in the case of the biotin-PEs of shorter chainlengths [C(12:0) and C(14:0)] is metastability of the fluid phase observed below the transition temperature. Considerable insight into the thermodynamic behavior of the biotin-PE dispersions can be obtained by comparison with corresponding data from the parent underivatized phosphatidylethanolamines. This is done below, in terms of the chainlength dependence and of the chainlength-independent end effects. The appropriate comparison is with the biotin-PEs in 1 M NaCl, because the surface electrostatics of these lipids are then screened almost fully, but without appreciable ion binding (cf. Cevc et al., 1980; 1981). Additionally, comparison is made between the transition temperatures of the biotin-PEs in the presence and absence of salt, in order to reveal the effects of surface electrostatics in the latter case. In this analysis, the different structures of the lyotropic phases of the biotin-PEs are to be borne in mind, in particular that the gel phases are interdigitated and the fluid phases in the absence of salt are micellar (Swamy et al., 1993). Thus both the chain packing and the surface area per lipid headgroup of the biotin-PEs can differ from those found with normal lamellar phospholipid phases.

Chainlength dependence of the calorimetric parameters

The chainlength dependences of the transition enthalpy, $\Delta H_t$, and the transition entropy, $\Delta S_t$, for the saturated diacyl biotin-PEs are given in Fig. 5, A and B, respectively. Only data from samples in 1 M NaCl are included. The data for samples in
the absence of salt are rather similar (cf. Table 1), but as noted above the transition curves are broad and more complex, and display metastability for the shorter chainlengths. Data for the corresponding underivatized PEs (average values from Marsh, 1990), are also included in the figure for comparison.

It is seen that, with the exception of DLBPE, the calorimetric values for both series of lipids have an essentially linear dependence on chainlength, \( n \), with constant end contributions, \( \Delta H_0 \) and \( \Delta S_0 \) (cf. Seddon et al., 1983):

\[
\Delta H_t = n \cdot \Delta H_{\text{inc}} + \Delta H_0 \tag{1}
\]

\[
\Delta S_t = n \cdot \Delta S_{\text{inc}} + \Delta S_0 \tag{2}
\]

where \( \Delta H_{\text{inc}} \) and \( \Delta S_{\text{inc}} \) are the incremental values per CH\(_2\) group. The values for the parameters obtained from the linear regressions to the calorimetric data shown in Fig. 5, A and B, are given in Table 2, which contains also some literature data for negatively charged phospholipids. The values for D LBPE were excluded from the linear regressions because of the uncertainties involved with the supercooling of water mentioned above.

Within certain limits, a linear chainlength dependence of the calorimetric parameters might be expected on a priori grounds (Cevc and Marsh, 1987), provided that the gel and fluid phases do not change appreciably with chainlength. With shorter chainlengths for which there is not such a clear distinction between chainlength-dependent and chain-end effects, or when the gel and fluid phases vary either in structure or in chain packing properties, deviations might possibly be expected. For the data in Fig. 5, this linearity approximately holds true and may be used as a first approximation to analyze (at least empirically) the effects of the different lipid headgroups on the chainlength-dependent and chainlength-independent contributions.
TABLE 2  Incremental values ($\Delta H_{inc}$, $\Delta S_{inc}$) of the chainlength dependence and end contributions ($\Delta H_o$, $\Delta S_o$) to the calorimetric enthalpy and entropy, respectively, for $N$-biotinyl phosphatidylethanolamines dispersed in 10 mM Hepes, 1 mM EDTA, pH 7.4 with or without 1 M NaCl, and underivatized phosphatidylethanolamines dispersed in $H_2O^*$

<table>
<thead>
<tr>
<th></th>
<th>$\Delta H_{inc}$ kcal/mol/CH$_2$</th>
<th>$\Delta H_o$ kcal/mol</th>
<th>$\Delta S_{inc}$ cal/mol/K/CH$_2$</th>
<th>$\Delta S_o$ cal/mol/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin-PE (1 M NaCl)</td>
<td>$1.07 \pm 0.04$</td>
<td>$-6.8 \pm 0.7$</td>
<td>$2.5 \pm 0.1$</td>
<td>$-7.5 \pm 2.5$</td>
</tr>
<tr>
<td>Biotin-PE (0 M NaCl)</td>
<td>$1.05 \pm 0.09$</td>
<td>$-6.5 \pm 1.6$</td>
<td>$2.3 \pm 0.4$</td>
<td>$-3.2 \pm 6.1$</td>
</tr>
<tr>
<td>PE*</td>
<td>$1.08 \pm 0.03$</td>
<td>$-9.0 \pm 0.6$</td>
<td>$2.8 \pm 0.1$</td>
<td>$-20.2 \pm 2.0$</td>
</tr>
<tr>
<td>PS§</td>
<td>$1.01 \pm 0.03$</td>
<td>$-5.1 \pm 0.4$</td>
<td>$2.5 \pm 0.1$</td>
<td>$-7.7 \pm 1.8$</td>
</tr>
<tr>
<td>PG*</td>
<td>$0.99 \pm 0.05$</td>
<td>$-5.2 \pm 0.6$</td>
<td>$2.6 \pm 0.2$</td>
<td>$-8.4 \pm 2.5$</td>
</tr>
</tbody>
</table>

* Obtained from the linear regressions given in Fig. 5 (cf. Eqs. 1 and 2).
‡ Average values from Marsh (1990).
§ Saturated symmetrical diacyl phosphatidyleserines from Marsh (1990).
* Saturated symmetrical diacyl phosphatidylglycerols from Marsh (1990).

The incremental values of the transition enthalpy and entropy are rather similar for the biotin-PEs and the underivatized PEs. This is as might be expected, since for both series of phosphatidylethanolamine lipids the transitions take place from an ordered gel to a fluid liquid-crystalline state of the lipid chains, although the overall structure of these phases is different in the two cases. The difference in the phospholipid headgroups is reflected, however, in the end contributions to the transition enthalpy and entropy. The value of $\Delta H_o$ is somewhat less negative for the biotin-PEs in 1 M NaCl than it is for the normal PEs. This is presumably because of the more open structure of the headgroup region in the interdigitated gel phase of the biotin-PEs, and possibly also because of stronger cohesive interactions between the biotin-PE headgroups. The value of $\Delta S_o$ is much smaller (although still negative) than that for the normal PEs, which suggests that the hydration of the biotin-PE headgroups is considerably different from that of the normal PEs (cf. Seelig, 1981). Both of these conclusions are in agreement with the interdigitated nature of the gel phase of biotin-PEs and the relatively hydrophobic character of the biotin moiety.

For comparison with negatively charged phospholipids, the data for a series of phosphatidylserines and phosphatidylglycerols are also given in Table 2, although the range of chainlengths covered is not as great as for the phosphatidylethanolamines. The incremental values of the transition enthalpy and entropy are again similar to those for the other phospholipids, as expected (cf. above). The end contributions to the transition enthalpy and entropy are closer to those of the biotin-PEs than they are to those of the normal PEs. This is most likely to be attributed to the higher degree of hydration of the negatively charged lipids than of normal PEs. It will be noted that, to within experimental error, there are not large energetic contributions to the thermodynamics of the chain-melting that arise from electrostatics because large differences are not seen between the biotin-PEs in the presence and absence of salt.

The gel-to-fluid phase transition temperatures for the saturated diacyl biotin-PEs of different chainlengths in 1 M NaCl and in the absence of salt are given in Fig. 6. For comparison, the data for saturated diacyl PEs of corresponding chainlengths taken from Seddon et al. (1983) are also included in the figure. The data from both systems are characterized by a steadily decreasing change in transition temperature with increasing chain length, as the contributions of the chains to the thermodynamics of the transition come to dominate over the end effects (cf. Marsh, 1991).

The chainlength dependence of the transition temperatures has been fitted to the expression predicted from the linear

![Figure 6](image-url)
dependences of the transition enthalpy and entropy given in Eqs. 1 and 2 (Seddon et al., 1983; Marsh, 1991):

\[
T_i = \Delta H_i / \Delta S_i = (\Delta H_{\text{inc}} / \Delta S_{\text{inc}}) \left[ 1 - (n_o - n_o') / (n - n_o') \right]
\]

(3)

where \( n_o \) (= -\( \Delta H_o / \Delta H_{\text{inc}} \)) and \( n_o' \) (= -\( \Delta S_o / \Delta S_{\text{inc}} \)) are the chainlengths at which the transition enthalpy and the transition entropy, respectively, extrapolate to zero. It is seen from Fig. 6 that the chainlength dependences of the transition temperatures of biotin-PEs in 1 M NaCl (and of saturated diacyl PEs in water) are depicted with good accuracy by Eq. 3. For biotin-PEs in the absence of salt, only the first three data points are fitted (the point for C(20:0) deviates from the trend), and therefore the parameters are not determined very sensitively. The fitting parameters for the saturated diacyl biotin-PEs in 1 M NaCl agree rather well with those deduced from the linear regressions to the calorimetric data given in Fig. 5. From Table 2, these are given by: \( \Delta H_{\text{inc}} / \Delta S_{\text{inc}} = 425.3 \) K, \( n_o - n_o' = 3.45 \), \( n_o' = 2.98 \). This gives further support for the method used previously to analyze the chainlength dependence of the transition temperatures for a wide range of different lipids (Marsh, 1991), including those with chainlength asymmetry (Marsh, 1992).

### Salt-induced transition temperature shifts

The decrease in transition temperature in the absence of salt is caused, at least in part, by electrostatic interactions arising from the charged biotin-PE headgroups (cf. Trauble et al., 1976). In 1 M salt, the electrostatic interactions are completely shielded, but dehydorization of the lipid headgroups does not occur until higher cation concentrations (Cevc et al., 1980; 1981). Assuming complete shielding at 1 M NaCl, the electrostatic contribution to the transition temperature in the absence of salt derived from double layer theory is (Trauble et al., 1976; Cevc and Marsh, 1987):

\[
\Delta T_i = -\frac{2RT}{\Delta S_i}(\Delta A/A)
\]

(4)

where \( \Delta A/A \) is the fractional change in the area per lipid molecule at the phase transition in the absence of salt, \( \Delta S_i \) is the transition entropy in 1 M salt, \( T \) is the absolute temperature, and \( R \) is the ideal gas constant. For the C(14:0) to C(18:0) biotin-PEs, the product of the experimentally measured values \( \Delta T_i \times \Delta S_i \) which is equal to the change in free energy at the phase transition from the additional (electrostatic) interactions (Cevc and Marsh, 1987), is approximately constant with a value of \(-350 \) cal/mol. From Eq. 4, this value corresponds to an increase in fractional area per lipid molecule of \( \Delta A/A = 0.29 \) at the phase transition. Whereas this value is comparable to that expected for the chain-melting phase transition of a normal lipid bilayer (cf. Marsh, 1990), it is surprisingly large for the biotin-PE system where the lipid chains are interdigitated in the gel phase (Swamy et al., 1993), even though the fluid phase in the absence of salt is micellar and therefore possesses a considerably more open headgroup structure. Possibly effects other than electrostatic interactions contribute also to the decrease of the phase transition temperature in the absence of salt. It will be noted that other repulsive headgroup interactions, and additionally changes in lipid headgroup hydration, would also predict that the area per lipid molecule must increase at the phase transition to account for a decrease in transition temperature (Cevc and Marsh, 1987). For C(20:0) biotin-PE, the salt-induced transition temperature shift is considerably smaller relative to the transition entropy (cf. Eq. 4) than for the biotin-PEs of shorter chainlengths, and correspondingly the predicted change in area per lipid molecule at the phase transition would also be smaller than the value quoted above, although still being an increase.

### Conclusions

The N-biotinylated phosphatidylethanolamines when dispersed in 1 M NaCl undergo sharp chain-melting transitions from ordered gel to fluid liquid-crystalline states whose calorimetric properties have a chainlength dependence similar to that for the underivatized phosphatidylethanolamines. Differences in the absolute values of the calorimetric parameters attributable to end effects give a measure of the interaction between the biotin headgroups and their change in hydration at the phase transition, which in turn are related to the different structures of the biotin-PE dispersions. These differences are then reflected in the respective transition temperatures. In the absence of salt, the transition temperatures of the biotin-PEs are lower, but the thermotropic phase behavior is considerably more complex and metastability is involved with the shorter chainlengths.

### REFERENCES


