NON-ELECTROSTATIC CONTRIBUTION TO THE TITRATION OF THE ORDERED-FLUID PHASE TRANSITION OF PHOSPHATIDYLGLYCEROL BILAYERS

Gregor CEVC*, Anthony WATTS and Derek MARSH

Max-Planck Institut für biophysikalische Chemie (Spektroskopie), D-3400 Göttingen Nikolausberg, FRG and *Institute for Biophysics and Institute J. Stefan, University E. Kardelj, 61000 Ljubljana, Yugoslavia*

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1. Introduction

The ordered-fluid phase transition of phospholipid bilayers is of interest not only because it yields information on the nature of the fluid bilayer regions in biomembranes, but also because it may provide a means for triggering lateral phase separation between the ordered and fluid lipids in membranes [1,2]. It has been shown [3–6] that the phase transition of negatively charged lipid bilayers shows strong temperature shifts on titrating the phosphate group of the lipid polar head. This provides a way of triggering the phase transition at constant temperature by varying the pH. The free energy of the bilayer made of protonated lipids, $G^* = H^* - TS^*$, changes upon deprotonation to $G^* - G^{\mathrm{pr}}$ and this change results in a shift of the phase transition temperature from $T_t^* = \Delta H_t^*/\Delta S_t^*$ to T_t, given by:

$$\Delta T_t = T_t^* - T_t = \Delta G_t^{\mathrm{pr}}/\Delta S_t^* \qquad (1)$$

where ΔH_t^* and ΔS_t^* are the transition enthalpy and entropy, respectively, of the protonated bilayer, and ΔG_t^{pr} is the change in G^{pr} at T_t. In principle, there are several possible contributions to G^{pr} and thus to ΔG_t^{pr}. ΔG_t^{el}, the electrostatic part of the ΔG_t^{pr} can be calculated within the frame of the Gouy-Chapman diffuse double layer theory [10]. In its high potential approximation, the corresponding electrostatic shift of the phase transition temperature of the lipid bilayer is then given by [4]:

$$\Delta T_t^{\mathrm{GC}} = (2kTN_{\mathrm{A}}/\Delta S_t^*)\,\Delta f/f - (4\epsilon\epsilon_0\,N_{\mathrm{A}}k^2T^2/e^2\Delta S_t^*)\,\Delta f\,\kappa \qquad (2)$$

Here, $\Delta f/f$ is the fractional change of area per charge (in the case of PG, also per molecule) at the phase transition; the reciprocal Debye screening length $\kappa = (2000\,N_{\mathrm{A}}e^2c/\epsilon\epsilon_0\,kT)^{1/2}$ is proportional to the square root of the bulk molar concentration, c (for 1:1 electrolyte c equals the ionic strength J), and all other symbols have their usual meaning [4].

In [3,4] it was suggested that the titration of the phase transition temperature of phosphatidic acid and methylphosphatidic acid bilayers could be accounted for solely in terms of the electrostatic surface energy of charged bilayers. Later, changes in hydrogen bonding between the lipid molecules have been proposed to contribute to the phase transition temperature shifts [7,8] and a difference in the angle of tilt of the acyl chains, between the protonated and non-protonated states, has also been postulated to shift markedly the phase transition temperature of lipid bilayers [9].

Here we have used the screening of the surface potential by monovalent ions to identify the electrostatic part of the phase transition shift in phosphatidylglycerol (PG) bilayers. This electrostatic contribution is found to be smaller than the total phase transition temperature shift on titration from pH 8 to pH 1.5 which we reported in [5] for complete protonation of the phosphatidylglycerol phosphate group. Independent evidence for the complete screening of the electrostatic part of ΔT_t is given by direct measurements of the electrostatic surface potential using charged spin labels. Thus assuming the salt-induced effects to be due principally to electrostatic screening, these experiments demonstrate the existence of a non-electrostatic contribution to the phase transition temperature shift on titration of phosphatidylglycerol bilayers.

2. Materials and methods

Dimyristoyl phosphatidylglycerol (DMPG) and dipalmitoyl phosphatidylglycerol (DPPG) were synthesized and characterized as in [5]. For measuring the electrostatic surface potentials, ψ_0, sonicated, ultracentrifuged dispersions of 5 mM DMPG were prepared in buffer (ionic strength $J = 0.05$) and spin labelled with cationic 4-(*N*,*N*-dimethyl-*N*-undecyl) ammonium-2,2,6,6-tetramethyl-piperidine-oxybromide [11] to yield SL/PG = 1/200 (mol/mol); the dispersions were split in half, one of which was saturated with NaCl (Suprapur, Merck, Darmstadt) and mixed with the other, to give samples of increasing ionic strength $0.1 < J < 2.3$. Samples were stored at 4°C, but never longer than 2 h, and the ESR measurements were performed on a Varian E-12 ESR spectrometer at (T_t–5°C).

Phase transition temperatures of multibilayer PG dispersions were determined by spin label partitioning using ESR [5] and by differential scanning calorimetry on a Perkin-Elmer model DSC-2. The scanning rates were 5 or 2.5°C . min^{-1}, and after the temperature runs the purity of the samples was confirmed by TLC to be >98%. Multilamellar dispersions of negatively-charged phospholipids take up water continuously in the absence of salt, but reach a limiting hydration in high salt concentrations [9,12]. In 1.5 M KCl at pH 8.0 the bilayer repeat distance for DPPG is 62 Å at 20°C [12].

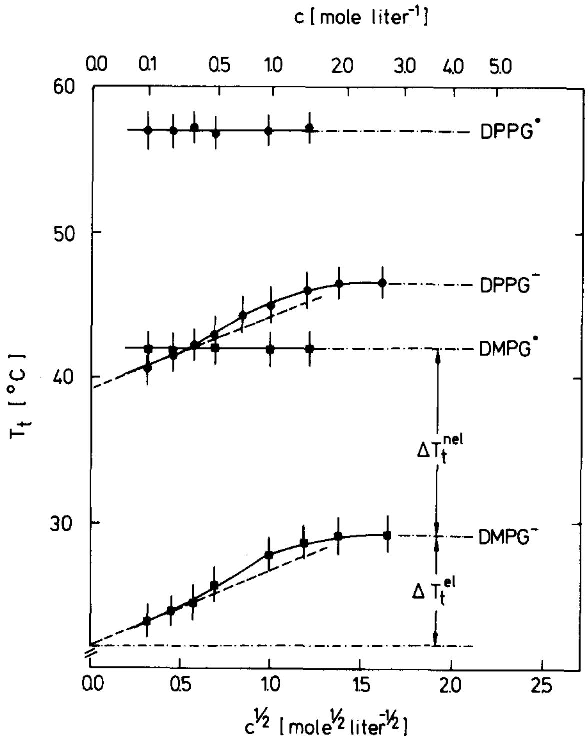

Fig.1. The ordered-fluid phase transition temperature, T_t, of DMPG and DPPG multibilayer dispersions as a function of the bulk monovalent ion concentration, *c*, and head group ionization. The buffers were 0.1 N HCl (pH 1.08 (PG^0) and triethanolamine · HCl/NaOH (pH 7.00) (PG$^-$) in which the ionic strength was adjusted by adding the appropriate ammounts of NaCl (Suprapur, Merck). The dashed lines were calculated from eq. (2) using $\Delta f = 0.11$ nm^2.

3. Results and discussion

The salt dependence of T_t for PG bilayers is given in fig.1. At ionic strength $J = 0.1$, the difference between the protonated state at pH 1 and deprotonated lipid state at pH 7 is $\Delta T_t = 18.7$ and 16.5°C for DMPG and DPPG, respectively. At pH 1, T_t is independent of ionic strength from $J = 0.1$–2.0, confirming that at this pH the PG molecules carry no net charge [5].

Eq. (2) predicts that the increase in T_t should depend linearly on the square root of the bulk ionic strength of the 1:1 electrolyte, i.e., $\Delta T_t \propto c^{1/2}$, for bilayers in the charged state. In fig.1, this relation is seen approximately to hold at low ionic strength, but at intermediate ionic strengths the screening is stronger than predicted by the simple electrostatic theory. At even higher ionic strengths, T_t flattens off to values of ~29°C for DMPG and ~46.5°C for DPPG. This suggests that the electrostatic phase transition temperature shift is effectively screened in 3 M NaCl, and that the total electrostatic contribution to the transition temperature shift is $\Delta T_t^{el} \simeq 6.5$ and 5.5°C for DMPG and DPPG, respectively.

Additional evidence for the complete screening of the electrostatic interactions at high ionic strengths comes from measurements of the bilayer electrostatic surface potential as a function of the bulk ionic strength. It is seen from fig.2 that the electrostatic surface potential approaches a limiting value, implying that it is fully screened at [NaCl]>2 M. (The surface potential axis in fig.2 is calibrated from the charged spin label partitioning assuming that $\psi_0 \simeq 0$ for [NaCl]>2 M [11].)

Interbilayer interactions are assumed not to make

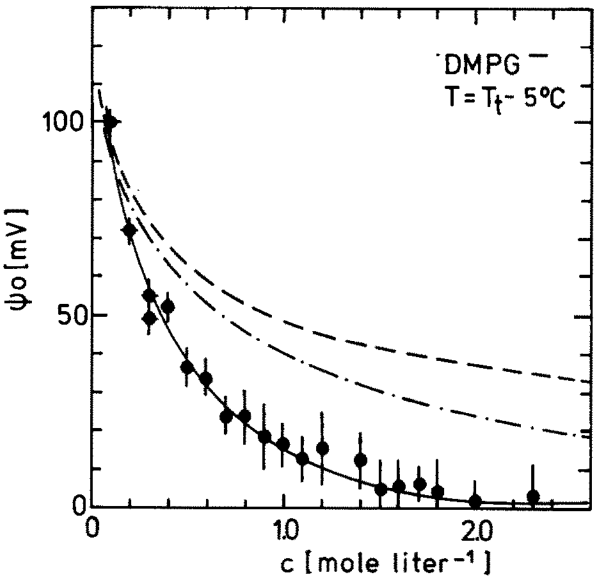

Fig.2. Salt dependence of the electrostatic surface potential ψ_0 of DMPG sonicated vesicles. ψ_0 was determined from the partition coefficient of a cationic amphiphilic spin label [11]. The dashed line represents the surface potential calculated from the Gouy-Chapman electrostatic theory assuming one charge per 1.2 nm² (120 Å²) (dashed-and-dotted line: the high potential approximation).

a large contribution to the salt-induced changes, since the effects are compensatory: The interbilayer spacing decreases with increasing salt in response to a decrease in the interbilayer repulsion. Also, electrostatic calculations indicate that the shift in T_t on bringing bilayers from infinite separation to close contact is <1°C for $\psi_0 \sim 30$ mV [13], and essentially no difference is observed in T_t of large multilamellar and single lamellar vesicles of the zwitterionic lipid phosphatidylcholine [14].

Neither the absolute values nor the salt dependences of the measured electrostatic effects of PG bilayers are wholely explicable in terms of straightforward Gouy-Chapman theory. Using the area/molecule $f \simeq 0.45$ nm² (45 Å²) from X-ray diffraction [12], the calculated electrostatic surface potential of PG bilayers at 0.1 ionic strength is $\psi_0^{GC} \simeq -149$ mV which is far larger than the experimental value $\psi_0 \simeq -99$ mV. Similarly, the electrostatic phase transition shift predicted by eq. (2) is $\Delta T_t^{GC} \simeq 17$°C for DMPG (13°C for DPPG) assuming an area change of $\Delta f/f \simeq 0.24$ at the phase transition and the calorimetrically measured values for the transition entropy of the protonated bilayers from table 1. (The value $\Delta f \simeq 0.11$ nm² (11 Å²) yielding $\Delta f/f \simeq 0.24$ was chosen to give the experimentally observed values of the initial gradient of the salt dependence of T_t in fig.1: $d\,\Delta T_t/d(c^{1/2}) \simeq 5$ and 4.5°C · mol$^{-1/2}$. litre$^{1/2}$ for DMPG and DPPG, respectively.)

If one assumes an artificially high area/molecule to fit the measured electrostatic surface potential—at $J = 0.1$ $\psi_0 \simeq \psi_0^{GC}$ implies that $f \simeq 1.2$ nm² (120 Å²)—the agreement is achieved for one particular ionic strength but the effect of the salt on ψ_0 is still not explained by simple Gouy-Chapman theory (see fig.2). Similarly, by taking $f = 1.2$ nm², the ΔT_t^{GC} values obtained from the eq. (2) become realistic, 6.5 and 5.5°C for DMPG and DPPG respectively, but the salt dependence of T_t remains anomalous. A dielectric constant of $\epsilon = 78$, corresponding to pure water, was used in these calculations. At 2 M NaCl, $\epsilon = 55$ [15], but this would only decrease the screening term in eq. (2) by ~15%. The use of the high potential approximation instead of the exact expression for ψ_0^{GC} also cannot account for this anomaly, as can be seen from fig.2. However, the agreement between the theory and experiment can be improved

Table 1
Transition temperature shifts and thermodynamic parameters of phosphatidyl-glycerol multibilayers

Lipid	ΔT_t (°C)	ΔT_t^{el} (°C)	ΔT_t^{nel} (°C)	ΔH_t^* KJ/mol (kcal/mol)	ΔS_t^* J/mol . K (cal/mol . K)	ΔT_t^{GC} (°C)	
						$f = 1.2$ nm²	$f = 0.45$ nm²
DMPG	18.7	6.5	12.2	23.1 (5.5)	73.5 (17.5)	6.5	17
DPPG	16.5	5.5	11.0	31.5 (7.5)	95.3 (22.7)	5.5	13

ΔH_t^* and ΔS_t^* are calorimetrically determined values of the transition enthalpy and entropy of multibilayer dispersions of protonated PG by refining the Gouy-Chapman theory with further assumptions, for example by considering the ion binding or the solvent effects.

In summary, several lines of evidence lead us to attribute the salt-induced changes principally to electrostatic screening.

(1) No salt dependence is seen in the uncharged state of the bilayer at low pH.

(2) The induced shifts saturate at a salt concentration coincident with that at which the surface potential is completely screened.

(3) The pH of the measurements is sufficiently far removed from the apparent $pK_a \sim 2.9$, that there will be no change in degree of ionization with increasing salt concentration [4,5].

Thus the salt dependence in fig.1 provides evidence for a non-electrostatic contribution, $\Delta T_t^{nel} = \Delta G_t^{nel}/\Delta S_t^*$, to the phase transition temperature shift caused by the lipid titration (see table 1). This non-electrostatic, i.e., non-screenable, shift may be due to the tilting of the acyl chains but other effects such as changes in the polar head group–water interactions or the strength of the hydrogen bonding, may also be important.

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