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ARTICLE

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# Modification of anomalous swelling in multilamellar vesicles induced by alkali halide salts

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**Abstract** By use of small-angle X-ray scattering it is shown that addition of alkali halide salts in small amounts (0–200 mM) shifts the repeat spacing in multilamellar $DC_{13}PC$ vesicles and alters the anomalous swelling behaviour close to the main transition. Both effects follow the Hofmeister series of the ions. We suggest that the shift of repeat spacing can be explained by ion effects on the van der Waals attractive forces between the membranes and on the decay length of the repulsive hydration force. The anomalous swelling is explained in terms of a critical unbinding of the membranes. The proximity of the critical temperature of the unbinding to the main transition temperature can be tuned by varying the concentration and type of salt in the sample.

**Keywords** Phospholipid bilayers · Salt · Hofmeister series · Small-angle X-ray scattering · Unbinding transition

## Introduction

There are two main reasons for investigating the effect of small amounts of salt on the swelling behaviour of vesicles close to the main transition temperature. First, the origin of anomalous swelling in multilamellar vesicles upon approaching the main transition (Hønger et al. 1994; Zhang et al. 1995) is still a matter of dispute. In one model the swelling takes place in the water layer between the membranes and can be described as the proceeding of a critical unbinding of the membranes (Hønger et al. 1994). In another, the anomalous swelling is mainly a bilayer thickening due to critical straightening of the hydrocarbon chains (Zhang et al. 1995). In yet a third proposition put forward recently the anomalous swelling is mainly a matter of precritical lipid headgroup hydration accompanied by increasing chain order (Richter et al. 1999). Chen et al. (1997) showed that the anomalous swelling primarily takes place in the water layer, but Nagle et al. (1998) argued that bilayer thickening accounts for some but not all of the observed swelling.

In an earlier study (Korreman and Posselt 2000) we varied the criticality of the main transition by changing the lipid chain length. The results thus obtained were in agreement with the predictions of the unbinding model with respect to the dependence of anomalous swelling on lipid chain length (Hønger et al. 1994). In the present study we investigate the predictions of the unbinding model with respect to variation of the interlamellar forces by adding salt to the aqueous solution, thus contributing further to a clarification of the nature of the anomalous swelling.

The second reason for the investigation is that the physiological condition of biomembranes is always a saline environment, and the salinity may be crucial for the functionality and behaviour of membranes. In some specific situations this is well understood, but often it is not. The system studied here is a very simple model system of a real biomembrane, and some of the basic mechanisms of salt effects on membrane behaviour may be studied this way.

In this paper it is shown that relatively small amounts (0–200 mM) of alkali halide salts have a huge impact on the swelling behaviour of vesicles. Anomalous swelling behaviour close to the main transition accommodates the possibility of precise thermal tuning of intermembrane interactions, and this possibility is even more powerful in the presence of salts because the range of attainable interactions is increased.

We suggest that we observe a critical unbinding of the membranes driven by thermal renormalization of the undulation repulsion between the membranes (Lemmich et al. 1995). The proximity of the critical temperature of

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the unbinding to the main transition temperature may be changed by addition of salts, through changes in the zero-frequency Hamaker constant and in the decay length of the hydration force (see below). The unbinding is not complete because of the intervention of the chain freezing at the main transition temperature. In principle, however, use of a sufficient amount of the proper salt could bring the complete unbinding within reach.

## Materials and methods

Phosphocholine lipid with two fully saturated identical fatty acid chains with a length of 13 methylene groups – ditridecanoylphosphatidylcholine, abbreviated $DC_{13}PC$ – was purchased from Avanti Polar Lipids and used without further purification. Fully hydrated samples of multilamellar vesicles were prepared in the following way. The lipid was dissolved in aqueous salt solution (water used was Milli-Q; resistivity 18 MΩ cm) in an amount corresponding to a lipid concentration of 20 wt%. The salt concentration ranged from 0 to 200 mM, and the salts used were KF, KCl, KBr, KI, NaF, NaCl and NaBr. The lipid solution was kept at a temperature approximately 10 °C above the main transition temperature for 2 h and vortex-mixed every 15 min. The main transition from the low-temperature solid ordered ripple phase ($P_{\beta'}$) to the high-temperature fluid disordered phase ($L_\alpha$) takes place at the temperature denoted $T_m$, which for $DC_{13}PC$ is 14.0 °C (Cevc 1993).

The samples were placed in a Lindeman quartz cuvette and mounted in a Kratky small-angle camera with a 1D detector (Hecus M.Braun-Graz) using Ni-filtered $CuK_\alpha$ radiation ($\lambda = 1.54$ Å).

Experimental conditions and data analysis have been thoroughly described previously (Korreman and Posselt 2000). We have estimated that geometric smearing of the spectra, due to the slit collimation of the Kratky camera, shift all repeat distance curves upwards by approximately $0.55 \pm 0.1$ Å, which should be taken into account when comparing the absolute repeat distance values of this study with others. Desmearing of very narrow Bragg peaks is not trivial, and we have therefore not performed desmearing of the spectra (Korreman and Posselt 2000).

At temperatures very close to $T_m$ it is not always possible to ascribe spectra unambiguously to either phase. In each measurement series we have therefore omitted the repeat distance data point closest to the main transition temperature from graphs and analysis.

For some samples (with KBr, NaBr and KCl), the ripple phase spectra looked very different from usual ripple phase spectra, and we have not attempted to index the peaks. Therefore we have not been able to extract the ripple phase repeat distance for these samples. Additionally, the Bragg peaks in the fluid phase broadened significantly close to $T_m$ for these samples.

## Theory

As mentioned above, we have earlier advocated the unbinding model for the anomalous swelling in multilamellar vesicles (Korreman and Posselt 2000). Within this framework, the anomalous swelling can be described by the power law (Lipowsky and Leibler 1986; Lemmich et al. 1995):

$$D - D_0 \propto (T - T_c)^{-1} \quad (1)$$

where $D_0$ is the repeat distance level well in the fluid phase, and $T_c$ is the critical temperature at which the bilayers unbind. Close to the main transition in multilamellar phosphatidylcholine vesicles, the temperature is the driving field for critical unbinding, because of a fluctuation-induced renormalization of the bending rigidity as the temperature approaches the main transition temperature from above (Lemmich 1996).

In general, a parameter that varies smoothly with temperature can be a driving field for critical unbinding, and one example of this is (Lipowsky and Leibler 1986):

$$D - D_0 \propto (W - W_c)^{-1} \quad (2)$$

where $W$ is the Hamaker constant of the van der Waals interaction between layers and $W_c$ is the critical Hamaker constant for bilayer unbinding, given by the bending rigidity of the bilayers, the membrane thickness and the parameters in the hydration force. Unbinding transitions of lipid bilayers have been observed by, for example, Mutz and Helfrich (1989) and Vogel et al. (2000), although in a somewhat different context.

The repeat spacing in a multilamellar vesicle is determined by the balance between four interlamellar forces: the van der Waals attraction, the hydration repulsion, the undulatory repulsion and the electrostatic double layer repulsion. Electrostatic double layer repulsion is usually disregarded for phosphatidylcholines in pure water [except for impurities in the lipid (Pincet et al. 1999)]. Although the zwitterionic nature of a surface under certain conditions (regular distribution of out-of-plane dipoles) produces a contribution to the electrostatic double layer repulsion (Israelachvili 1992, pp. 254–256), those conditions are not met by phosphatidylcholine surfaces in the fluid phase. In a solution containing ions, there may be electrostatic interactions due to binding of the ions to the lipids. Of the ions used here, only $I^-$ binds appreciably to PC head groups (Marra and Israelachvili 1985; Macdonald and Seelig 1988; Clarke and Lüpfert 1999). In the present study, we observe that the scattering spectra and repeat distance versus temperature curves for samples containing KI are different from those for samples with the other salts. Furthermore, if the amount of KI exceeds approximately 10 mM, the macroscopic phase behaviour – the colour and texture – of the samples completely change, and the spectra show no Bragg peaks. We therefore assume that there is a non-negligible contribution from electrostatic double layer forces for samples with $I^-$, and we will not consider these samples any further. For all other salts investigated, we assume that there is no contribution from electrostatic double layer forces, i.e. the repeat distance is determined by the remaining three interlamellar forces.

For bilayer distances $l$ up to ca. 4–5 nm, the van der Waals potential can be described by the half-space approximation (Lipowsky 1995), involving two layers of infinite extent, infinite thickness and uniform density:

$$V_{vdW}(l) \approx -\frac{W}{12\pi}\frac{1}{l^2} \quad (3)$$

where $W$ is the Hamaker constant, consisting of a contribution from the static (zero-frequency) polarizability of the molecules in the solution ($W_{\nu=0}$) and a contribution from polarizabilities at finite frequencies. The van der Waals attraction will be reduced by the presence of salts owing to Debye screening of the zero-frequency contribution to the Hamaker constant given by (Mahanty and Ninham 1976):

$$W_{\nu=0}(l) = W_{\nu=0}(0)2\kappa l e^{-2\kappa l}; \quad \kappa l \gg 1 \tag{4}$$

where $W_{\nu=0}(0)$ is the unscreened Hamaker constant and $1/\kappa$ is the Debye screening length. The Debye screening length depends on the valence and the concentration of the ions in the solution, but not on the specific ion species, and is given as (Mahanty and Ninham 1976):

$$\frac{1}{\kappa} = \frac{0.304}{\sqrt{[\text{salt}]}} \text{ nm} \tag{5}$$

for monovalent salts, where [salt] denotes the molar salt concentration. The screening length of pure water is approximately 1 μm (Israelachvili 1992, p 238), and thus the presence of 100 mM of a 1:1 electrolyte decreases the screening length by a factor of ca. 1000. Addition of salts will thus affect the anomalous swelling behaviour through a screening reduction of the Hamaker constant. Assuming that the critical Hamaker constant is unchanged, addition of a salt will cause $W$–$W_c$ at a given temperature (and thereby $T$–$T_c$) to decrease, in effect enhancing the anomalous swelling.

Although the Debye screening length is not dependent on ion species, there is still a possibility of an ion specificity in the screening of the Hamaker constant, if there is a difference between the concentrations of the different salts in the interlamellar water. Such a difference would arise if the differences of ion solubilities in the excess water and the interlamellar water, respectively, are very different for different ion species. Differences between anion and cation solubilities for the salts will, however, be counteracted by the tendency to keep the electric potential neutral. Measurements of, for example, changes in phase transition temperatures reported in the literature are indicative of an uneven distribution of ions between interlamellar and bulk water (see, for example, Koynova et al. 1997). However, to our knowledge, large variations in the difference between solubilities in bulk water and interlamellar water of different alkali metal and halide ions are not reported in the literature, and, as will be discussed in the next section, it is unlikely that such differences can account for the large degree of ion specificity that we observe.

There is no reason to expect that ions dissolved in the interlamellar water, but not binding to the membranes, should affect the bending rigidity of the bilayers significantly. We will therefore not consider changes in the undulation force further.

This leaves the repulsive hydration force to account for possible ion specificity of the effects, which we will return to below. A treatment of the effect of ions on the hydration force is not trivial, because the very nature of the hydration force is still a puzzle.

## Results

In Fig. 1, the repeat distance versus temperature is shown for suspensions of vesicles in pure water and in aqueous solutions with 100 mM salt concentration. All salt curves except that for NaCl exhibit a shift of the repeat distance well in the high-temperature fluid phase with respect to the repeat distance of the pure water suspension, and the value of the shift is clearly ion specific. The "order" of the effect of salts on this shift (see Fig. 1) is:

$$\text{NaCl} < \text{KF} < \text{KCl} < \text{NaF} < \text{NaBr} < \text{KBr} \tag{6}$$

All show a higher repeat distance than vesicles in pure water, except the sample with NaCl, which shows a repeat distance equal to that of the pure water sample within the experimental uncertainty. KBr gives the largest shift to higher repeat distance with a value of 8.2 Å, or almost 14% of the repeat distance of the pure water sample. This effect of KBr on the repeat distance has been reported before (Cunningham and Lis 1986; Cunningham et al. 1986), but a systematic study of different salts in different concentrations and in a temperature interval has not been performed previously. Although the curves cannot be said to have reached constant levels within the measured temperature interval, the shift of repeat distance at high temperatures signifies a displacement in the balance of the interlamellar forces.

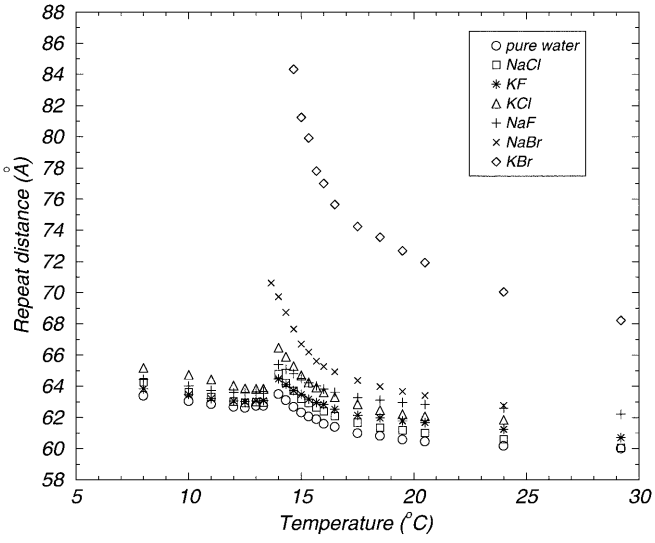


**Fig. 1** The repeat distance versus temperature for $DC_{13}PC$ vesicles in pure water and in 100 mM concentrations of salts. For $Br^-$ salts, we were not able to index the ripple phase, and thus there are no data below $T_m$. In spectra very close to $T_m$ it was often difficult to read a reliable peak position, and consequently points have been omitted

Furthermore, the presence of salt has in all cases an effect on the anomalous swelling upon approaching the main transition temperature in the fluid phase. It can be observed from Fig. 1 that the effect of the salts on the anomalous swelling (defined as the difference between the repeat distance at the transition temperature and at the highest temperature in the fluid phase) follows a slightly different order from the one given in Eq. 6:

$$KF < NaF < NaCl < KCl < NaBr < KBr \quad (7)$$

Also in this respect, KBr has the biggest effect, with an anomalous swelling of more than 16 Å, or almost 24% of the repeat distance value, well in the fluid phase. This amount of swelling is much larger than what can be explained by increases in bilayer thickness and headgroup hydration alone; thus swelling in the water layer between the membranes must play a significant role.

A repeat distance shift and an increased anomaly is expected owing to the Debye screening reduction of the Hamaker constant as described above. The large degree of ion specificity, however, is not immediately expected. Supposing that Debye screening of the Hamaker constant alone should account for the shifts in repeat distance, the lack of shift in the repeat distance for the NaCl sample would mean that there are no ions in the interlamellar water of this sample.

Considering this, we find it unlikely that the ion specificity in the effect of salts on the swelling behaviour is solely due to concentration differences. Since we have earlier ruled out contributions from the electrostatic double layer force and the undulation force, this leaves the hydration force to be considered.

It is well known that, at small distances from a hydrophilic surface, there is a strong repulsive potential that can be described by an exponential:

$$V_{\mathrm{hyd}}(l) \propto \mathrm{e}^{-l/\lambda_{\mathrm{hyd}}} \quad (8)$$

where $\lambda_{\mathrm{hyd}}$ is the decay length of the hydration force away from the hydrophilic surface (Lipowsky 1995); $\lambda_{\mathrm{hyd}}$ is typically in the order of 1–3 Å. For a long time it has been believed that the hydration force is a result of a perturbation of the structure of water around hydrophilic polar surfaces. This has been described very thoroughly in several papers (e.g. Rand and Parsegian 1989; Leikin et al. 1993). Many attempts have been made to set up formal models for this, one of them described by Marčelja and Radic (1976), but so far none of the suggested models have prevailed. Recently, it has been suggested that the hydration force may in fact be a matter of protrusion of single molecules from the surfaces, producing a steric force, which was shown to exhibit exponential behaviour (Israelachvili and Wennerström 1992). In this paper we choose to adhere to the former picture, because it is still the most prevalent. Also, we find that there are important arguments against the latter picture, which have not (yet) been refuted (Parsegian and Rand 1995).

Within the notion of a force arising from perturbation of water structure, it is not difficult to imagine that addition of ions can change the value of the decay length $\lambda_{\mathrm{hyd}}$, because ions perturb water structure as well. We suggest the following mechanism, in keeping with a notion briefly outlined in Marra and Israelachvili (1985).

Ions that hydrate strongly compete with the lipid headgroups for water molecules, and make the water less available to the headgroups. This may be termed a salting-out effect, and would tend to reduce the range of the hydration force. The opposite would happen for ions that hydrate weakly: they make the water molecules more available to the lipid headgroups (they salt in), and thereby tend to increase the range of the hydration force. In other words, addition of ions, for which the mobility of adjacent water molecules is low (also termed water-structure makers), will cause the decay length of the hydration force to decrease. Addition of ions that induce high mobility of adjacent water molecules (termed water-structure breakers) will increase the decay length. When the decay length of the hydration force is changed, the value of the critical Hamaker constant is changed (Lipowsky and Leibler 1986). A higher decay length, which extends the range of the hydration repulsion and thereby displaces the balance of the interlamellar forces in a repulsive direction, will increase $W_c$, bringing $W_c$ closer to $W$ if all other conditions are left unchanged. According to our proposition, when a water-structure-breaking salt is added to a sample, this is exactly what happens, at the same time as the value of the Hamaker constant $W$ is lowered owing to Debye screening, i.e. the interlamellar repulsion increases and the criticality of the unbinding increases.

In effect, addition of a water-structure-breaking salt will, at any given temperature $T$, bring $T_c$ closer to $T$. Because of the intervention of the chain freezing main transition at $T_m$, which stops the critical unbinding transition proceeding, it is not possible to measure the value of $T_c$ directly. However, when $T_c$ is changed, the value of $T_m$–$T_c$ may be changed, since $T_m$ is not altered much by the presence of 100 mM alkali halide salts (we have measured, by use of differential scanning calorimetry, that $T_m$ changes less than 0.2 °C). The presence of a water-structure-breaking salt will decrease $T_m$–$T_c$. Addition of a water-structure-making salt will decrease $W_c$ while also decreasing $W$, and depending on which effect is strongest, it can either increase $T_m$–$T_c$ opposite to a water-structure-breaking salt or decrease $T_m$–$T_c$.

Solutes are often ranked in the Hofmeister series, which reflects the interactions of solutes with water and/or various macromolecules (Collins and Washabaugh 1985). Although most experiments yield identical Hofmeister orderings, there are some discrepancies in the literature concerning cations. Here we define the Hofmeister series in terms of the water structuring capacity, i.e. the hydration properties, which according to Samoilov (1972) is as follows for the monovalent alkali metals and halides:

$$Li^+ > Na^+ > K^+ > Cs^+ \quad (9)$$

$$F^- \gg Cl^- > Br^- > I^- \quad (10)$$

with turning points around $Na^+$ and $Cl^-$, respectively (Collins and Washabaugh 1985), such that ions to the right are water-structure breakers. It is generally acknowledged that the perturbing effect of anions is greater than that of cations (Conway 1978). Except for the fluoride salts, the order of the effect of salts on the repeat distance shift is well in accordance with the order of the Hofmeister series of the ions (taking into account that the effect of anions is greatest). From the Hofmeister series we would have expected the samples containing NaF and KF to exhibit shifts smaller than those of the other samples. Instead, they are to be found in the middle of the series, and with the order of NaF and KF switched. The effect of the salts on the amount of anomalous swelling follows the Hofmeister series, except that also here the order of NaF and KF is switched. For all salts without $F^-$, the larger the upwards shift of the repeat distance level, the more enhanced is the anomalous swelling.

We find that the inability of $F^-$ salts in vesicle samples to fit into the Hofmeister systematics, stated above, does not constitute a fundamental problem in our proposition for the effects of salts. As stated, it is possible for a water-structure-making salt to increase $W$–$W_c$ (if $W_c$ is reduced more than W) while increasing also the repeat distance level in the fluid phase (if the effect of Debye screening on the repeat distance is large compared to that of reduction of the hydration decay length). A large Debye screening could, for example, be a result of a large interlamellar salt concentration of the fluoride salts. We have not been able to find literature to support this, but literature concerning $F^-$ in phospholipid vesicles is generally very scarce. It is a fact, however, that $F^-$ interacts strongly with water compared to the other halides, and is known to produce basic aqueous solutions (as opposed to the rest of the ions used in this study, which are neutral) (Atkins and Beran 1992).

Of the alkali halide salts, there is no stronger water-structure maker according to the Hofmeister series than NaF [$Li^+$ has been suggested to bind significantly to phosphatidylcholine (Cunningham and Lis 1986) and is therefore not considered here]. However, preliminary data from a sample of $DC_{14}PC$ (DMPC) vesicles with $NH_4Cl$, which is another strong water-structure maker, were in full accordance with our hypothesis (unpublished).

We fit the power law in Eq. 1 to the data points above $T_m$ with $T_c$, $D_0$ and a constant of proportionality as free parameters. The results of the fits are shown in Table 1, and plots of the repeat distance versus $(T–T_c)^{-1}$ are shown in Fig. 2 ($Na^+$ salts) and Fig. 3 ($K^+$ salts), together with the fitted curves (the solid straight lines in the figures). The systematics of the fits are in accordance with the prediction of the unbinding model, assuming our suggestion for the ion effect on the hydration force.

**Table 1** Results of power law fitting, showing the main transition temperature and the three fitting parameters, $T_c$, $D_0$ and $k$, for all samples. The main transition temperature has been found by use of the scattering spectra

| Salt | Concentration (mM) | $T_m$ (°C) | $T_m$–$T_c$ (°C) | $D_0$ (Å) | $k$ |
|---|---|---|---|---|---|
| Pure water | – | 13.8 | 2.2 | 59.4 | 9.9 |
| KF | 100 | 13.5 | 2.8 | 60.1 | 14.8 |
| NaF | 100 | 13.8 | 2.2 | 61.8 | 9.2 |
| NaCl | 100 | 13.5 | 2.0 | 59.4 | 13.5 |
| KCl | 100 | 13.5 | 1.5 | 60.7 | 11.9 |
| NaBr | 100 | 13.5 | 1.1 | 61.7 | 13.4 |
| KBr | 100 | 13.5 | 0.9 | 67.0 | 34.9 |

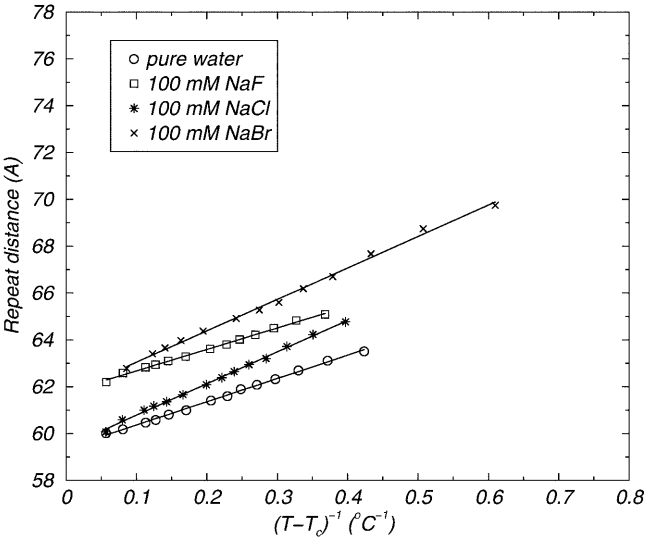


**Fig. 2** The repeat distance versus $(T–T_c)^{-1}$ (where $T_c$ is a fitting parameter) for all $Na^+$ salts in 100 mM concentration

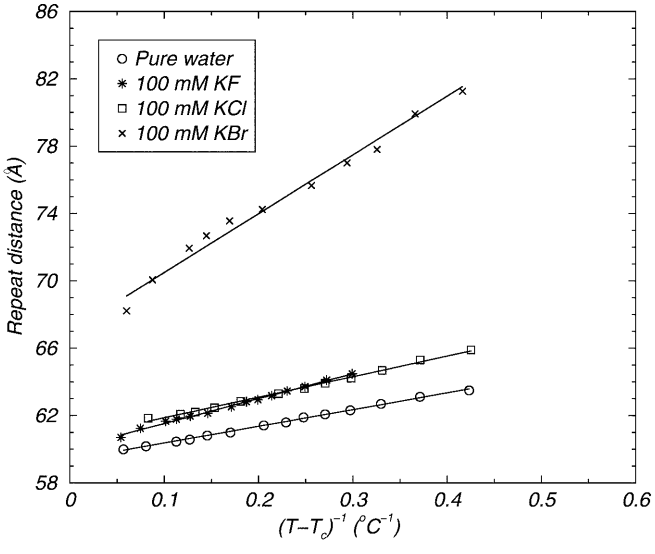


**Fig. 3** The repeat distance versus $(T–T_c)^{-1}$ (where $T_c$ is a fitting parameter) for all $K^+$ salts in 100 mM concentration

The repeat distance level in the fluid phase, $D_0$, increases for all salts with a systematism similar to the order of the shift given in Eq. 6.

The proximity of the main transition temperature to the critical temperature at which the bilayers completely unbind increases the stronger is the water-structure-breaking capacity of the added salt. The water-structure-making salts (containing $F^-$) either increase $T_m$–$T_c$ or leave it unchanged. In Figs. 2 and 3 it can be seen that the data points are fairly well scattered around the straight lines of the fits. The only points that differ significantly from straight lines are the ones for $Br^-$ samples, for which we had difficulties reading the peak position of the Bragg peaks when approaching the main transition. The peaks broadened and their intensity decreased. In Fig. 4 we have shown spectra for vesicles in 100 mM KBr to illustrate the difficulty in reading the peak positions.

The small temperature range and the amount of noise in the data do not allow for a determination of the true value of the critical exponent in the power law (and none of the restraints can be significantly overcome experimentally). Since the power law fitting is very sensitive to small changes in the parameters, neither $T_c$ nor $D_0$ can be eliminated as free parameters by estimation. In Richter et al. (1999), the value of the critical exponent was found to be close to 0.02, when $D_0$ was chosen to be zero. However, this choice of $D_0$ is not appropriate, since the minimum repeat distance must at least be the bilayer thickness.

We here conclude that choosing a value of 1 for the critical exponent gives good fits, and furthermore that the systematism with respect to the presence of salts in the samples is in accordance with the notion of the anomalous swelling as a critical unbinding phenomenon.

In Fig. 5 the repeat distance versus temperature is shown for different concentrations of NaBr. The repeat distance in the fluid phase far from the transition temperature seems to increase with salt concentration in a simple manner. The shifts in repeat distance from 0 to 100 mM NaBr and from 100 to 200 mM NaBr are approximately the same. The anomalous swelling, however, is inhibited close to the main transition temperature for the high salt concentrations. This could be an effect of structural restrictions on the swelling behaviour due to the closed topology of multilamellar vesicles.

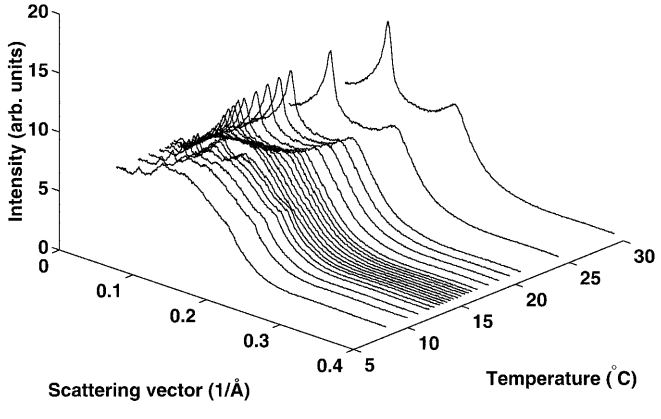


**Fig. 4** Spectra for all temperatures for $DC_{13}PC$ vesicles in 100 mM KBr solution. It is seen that in the fluid phase close to the main transition the peaks broaden and the intensity decreases. In the ripple phase we have not tried to index peaks, because the spectra are very different from usual

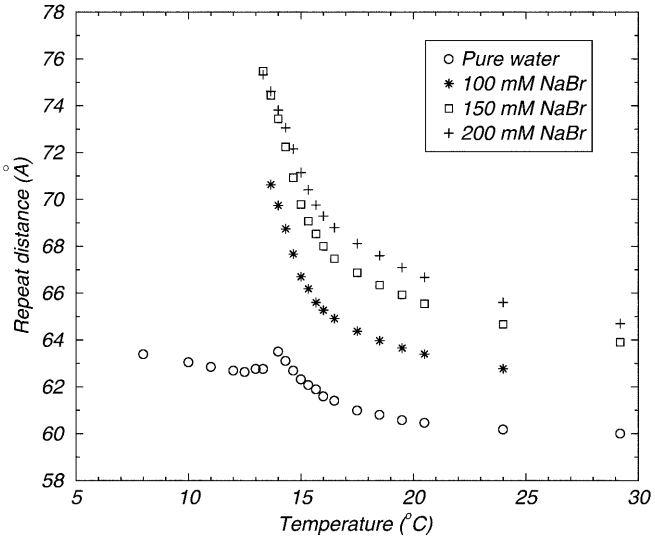


**Fig. 5** The repeat distance versus temperature for $DC_{13}PC$ vesicles in pure water and three concentrations of NaBr. In the ripple phase we were not able to index the spectra, when salt was present, so there are only points in the fluid phase

An interesting feature is that both the peak positions and intensities of the spectra of the ripple phase change very much with addition of certain salts (NaBr, KBr and sometimes KCl). We have in one case attempted an indexing of the peaks and this suggested a ripple phase with a monoclinic lattice cell with an acute angle and a short ripple periodicity. Effects of salt on the ripple phase have been reported before (Tölgyesi et al. 1985). It would be interesting to relate this phenomenon to the newly proposed model for the ripple phase, which relates the rippling to periodically arranged gel and fluid domain formation (Heimburg 2000). We do not observe any Bragg peak "splitting" in the fluid phase as reported by Rappolt et al. (1998) in the presence of alkali halides, although the salt to lipid concentrations at least in one instance (KCl) are comparable. The primary difference between the samples used in this work and by Rappolt et al. (1998) is that we use a lipid with a shorter chain length.

## Discussion

We have shown that addition of alkali halides in small concentrations to multilamellar vesicles has a big impact on their swelling behaviour. The effect follows the Hofmeister series of the ions, excepting for salts

involving $F^-$. In the presence of $I^-$, which binds to the bilayers, significant electrostatic double layer forces are introduced, and we have therefore not attempted analysis of our data involving salts containing $I^-$.

We suggest that the anomalous swelling can be described in terms of a critical unbinding, and that the effect of alkali halides on the swelling behaviour can be explained by a Debye screening reduction in the Hamaker constant combined with a salt-induced change of the hydration force decay length. Water-structure-breaking salts tend to increase the decay length, whereas water-structure makers tend to decrease the decay length.

According to the theory of critical unbinding, the repeat distance exhibits a power law dependence on the Hamaker constant, with a critical Hamaker constant determined by the bending rigidity and the parameters in the hydration force (Lipowsky and Leibler 1986). The critical exponent of unbinding is close to one. We have assumed this model to be correct and have tested the predictions of the model regarding the addition of salt. When salts are added, the proximity of the Hamaker constant to the critical Hamaker constant is changed. When a water-structure-breaking salt is added, the critical Hamaker constant is brought closer to the Hamaker constant of the solution, and when a water-structure-making salt is added, the critical Hamaker constant is decreased. The Hamaker constant of the solution is decreased by the addition of all salts, owing to Debye screening. Close to the main transition, the temperature is the driving field of the critical unbinding through a renormalization of the bending rigidity (Hønger et al. 1994), and thereby the critical Hamaker constant. In effect, the addition of salts is predicted to change the proximity of the main transition temperature to the critical temperature of unbinding: a reduction in $W–W_c$ corresponds to a reduction of $T_m–T_c$. By fitting to the power law in Eq. 1, we observe that addition of water-structure-breaking salts indeed reduces $T_m–T_c$. We also observe that addition of water-structure-making salts tends to increase $T_m–T_c$, but at the same time they increase the repeat distance.

In principle, the critical Hamaker constant could be changed so much by the addition of salts that the critical unbinding observed for $T \rightarrow T_m$ would become complete. In practice, this will not happen owing to the geometric restriction of the closed vesicles. If complete unbinding should occur, the vesicles would break up.

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