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Rapid report

Slow relaxation of the sub-main transition in multilamellar phosphatidylcholine vesicles

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Abstract

The influence of ionic strength and equilibration time on the appearance of the sub-main transition in fully hydrated multilamellar vesicles composed of phosphatidylcholines has been investigated by means of calorimetry and densitometry. The heat capacity measurements show that the transition enthalpy of the sub-main transition is affected by both salt concentration (KCl) and equilibration time. The small heat capacity peak appearing in vesicles made in pure water is significantly increased upon addition of salt. Furthermore, equilibration of the multilamellar vesicles at low temperatures for several weeks results in a pronounced enhancement of the transition enthalpy of the sub-main transition. Neither salt concentration nor equilibration time affected the transition temperature of the sub-main transition. In the densitometry measurements a small volume change is detectable for high salt concentrations. In order to gain further insight into the physical mechanisms involved in the sub-main transition, a Monte Carlo computer simulation study has been carried out using a microscopic model. The combined experimental and simulation results suggest that the sub-main transition involves an acyl chain disordering of phospholipids in lipid bilayer regions that are characterized by a locally decreased lateral pressure most likely caused by a curvature stress.

Keywords: Vesicle; Sub-main transition; Phosphatidylcholine; Calorimetry; Densitometry; Computer simulation

Multilamellar vesicles composed of saturated phospholipids can undergo several thermotropic phase transitions which are considered to be of relevance for biological membranes [1,2]. The most extensively studied transition is the chain melting main transition, which takes the bilayer from a low temperature gel phase with ordered acyl chains to a high temperature fluid phase characterized by highly disordered acyl chains [2,3]. In addition to the main transition, saturated diacyl phosphatidylcholine bilayers display thermotropic phase transitions such as the sub-transition and the pre-transition. The sub-transition is a low-temperature acyl chain packing transition involving slow kinetics and an equilibration time of several weeks for the formation of a crystalline-like lipid bilayer phase [4]. The pre-transition, which takes place a few degrees below the main transition, involves a two-dimensional reorganization of the lipid bilayer and the formation of a

Abbreviations: DC_nPC, saturated diacyl phosphatidylcholine with n carbon atoms in each acyl chain; DSC, differential scanning calorimetry

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lateral bilayer structure with long range order known as the ripple phase [5].

Recently, thermodynamic measurements have identified a highly cooperative low-enthalpy transition taking place close to the main transition of fully hydrated long chain phosphatidylcholine bilayers [6,7]. A two-stage melting process involving a lateral lipid disordering at slightly lower temperatures than the lipid acyl chain melting has been proposed to explain the appearance of a minor endotherm before the major endotherm of the main transition [8]. However, a detailed understanding of the molecular mechanisms involved in the sub-main transition is still lacking although simultaneous small- and wide-angle X-ray studies have demonstrated a change in the lipid acyl chain packing properties at temperatures corresponding to the small endotherm [9]. In the same study it was shown that salt significantly enhanced the heat capacity peak and the associated transition entropy excluding the possibility of a lateral disordering of the lipids [8,9]. In a recent study, it was furthermore demonstrated that addition of solutes to both uni- and multilamellar vesicles influenced the appearance of the heat capacity peak, and it was argued that the creation of osmotic stress across the lipid bilayer led to a change in some lipid packing parameters and a discrete shift in the chain melting temperature [10]. However, in order for this to be the case the presence of osmotically active agents in the aqueous phase is required.

By means of differential scanning calorimetry and densitometry, yielding information about enthalpic and volumetric changes of lipid bilayer phase transitions, we have made a systematic investigation of the influence of ionic strength and equilibration time on the appearance of the sub-main transition in long-chain phosphatidylcholine ($DC_{18}PC$, $DC_{19}PC$) multilamellar vesicles. In particular, we have investigated the appearance of the sub-main transition in multilamellar vesicles made in highly purified water excluding the possibility of any solute induced osmotic stress effects as earlier suggested [10].

All the phosphatidylcholine lipids used in the experiments were purchased from Avanti Polar Lipids (Birmingham, AL, USA). The multilamellar vesicles were made by dispersion of weighed amounts of lipids in milli-Q water containing 0, 50, 150 or 300 mM KCl as earlier described [6]. After preparation, the lipid samples for the calorimetric experiments were scanned immediately or after storage for 3 days or 10 weeks in the freezer at −20°C. Before use the frozen lipid suspensions were melted at room temperature. Heat capacity curves were obtained using a MicroCal MC-2 (Northampton, MA, USA) ultra-sensitive power compensating calorimeter equipped with a nanovoltmeter [6]. Density measurements of the multilamellar vesicle suspensions were obtained using an Anton Paar DMA 602 (Linz, Austria) vibrating tube densitometer allowing automated discrete temperature changes. Lipid samples for the volumetric experiments were used immediately after preparation. The volumetric measurements were obtained using 2.5 ml of 50 mM multilamellar vesicles. Data sampling was performed with a temperature increase of 0.05°C using an equilibration time of 1800 s at each temperature. The resolution of the densitometric method is about 2×10^{-6} cm^3/g, e.g., at a lipid concentration of 5% (w/w) a sharp transition will be detectable if it involves a change in the volume of the vesicles of about 10^{-4} cm^3/g.

In order to gain further insight into the physical mechanisms involved in the sub-main transition, a Monte Carlo computer simulation study has been carried out using an extended version of a microscopic model of the chain melting transition of saturated phospholipid bilayers [11,12]. The microscopic model, which is a multistate lattice model, is primarily based on the acyl chain conformational statistics of the main transition and contains several terms which in a detailed manner describe the internal energy and the interaction between the different conformational states of the acyl chains. The model also includes an intrinsic lateral pressure term, $\Pi = 30$ dyne/cm, which is incorporated in order to assure bilayer stability. Details of the microscopic model have earlier been described [12]. Within the model, the intrinsic lateral pressure Π represents the stabilizing forces in lipid bilayer formation. In particular, Π covers the balance between the polar head group and the acyl chain intrinsic pressure [11,12]. In a lipid bilayer of high curvature the outer part of the bilayer would experience a shift of this balance towards a lower interfacial pressure in the head group region. To a first approximation the effect of having an increased lipid bilayer curvature can be mimicked within the model as a corresponding decrease in Π. In the

Monte Carlo computer simulations we have studied the effects of a locally decreased lateral pressure corresponding to $\Pi = 27.5$ dyne/cm in a minor part of the lipid bilayer. This type of modelling has earlier been successfully used to investigate the relationship between curvature induced temperature shifts of phase boundaries of solid supported lipid bilayers characterized by different curvatures [13].

Fig. 1A shows differential scanning calorimetric results obtained at a scan rate of 4°C/h for pure one-component multilamellar $DC_{18}PC$ vesicles made in pure water. The C_p curve clearly demonstrates the existence of the small endotherm associated with the sub-main transition positioned just below the well-known main transition. The simple fact that the sub-main transition is present in the multilamellar vesicles made in pure water excludes the possibility of a solute induced osmotic stress effect as earlier argued [10]. The small endotherm of the sub-main transition, which is characterized by a C_p curve with a narrow half-height width of $\Delta T_{1/2} \sim 0.15$°C for $DC_{18}PC$ vesicles indicates a high degree of cooperativity similar to the transitional characteristics of the chain melting main transition [14,15].

The specific volume, V, of $DC_{18}PC$ multilamellar vesicles in a temperature interval of 5°C around the main transition is shown in Fig. 1B. The densitometric measurements reveal a large specific volume change, ΔV, involved in the main transition of the

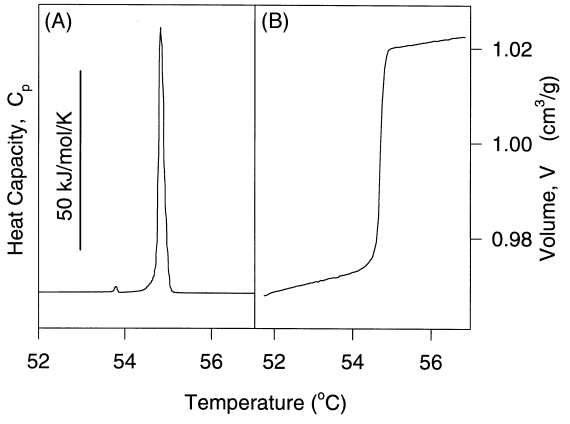

Fig. 1. (A) Heat capacity, C_p, of $DC_{18}PC$ multilamellar phospholipid bilayers obtained by differential scanning calorimetry at a scan rate of 4°C/h. (B) The specific volume, V, of $DC_{18}PC$ multilamellar vesicles as measured by densitometry. The temperature interval between each data point is 0.05°C.

$DC_{18}PC$ vesicles [14]. However, no volume changes could be detected at temperatures (cf. Fig. 1A) corresponding to the sub-main transition for vesicles prepared in pure water. Similar volume data were obtained for $DC_{14}PC$ to $DC_{20}PC$ phosphatidylcholine vesicles (data not shown). The lack of any sign of the sub-main transition thus suggests that the volume change of the sub-main transition is well below 1% of ΔV for the main transition. On basis of the densitometry results it is therefore impossible to determine whether the sub-main transition for vesicles made in pure water involves a volume change or simply is below the detection limit. Future high pressure heat capacity measurements obtained on vesicles are likely to provide deeper insight into small volume changes involved in the sub-main transition, i.e. a pressure induced temperature rise of the heat capacity peak. The heat capacity curves in Fig. 2 represent upscans of $DC_{18}PC$ and $DC_{19}PC$ vesicles which are freshly made in pure water or have been stored for either 3 days or 10 weeks. The data clearly reveal that the longer the vesicles have been stored the more pronounced does the endotherm of the sub-main transition become, indicating that the kinetics involved in the formation of the sub-main transition is very slow. Consecutive upscans (data not shown) of the vesicles stored for 10 weeks result in heat capacity curves similar to the curves for the freshly prepared vesicles as shown in Fig. 2A.

The calorimetric and densitometric measurements shown in Fig. 3 illustrate upscans of freshly made $DC_{18}PC$ vesicles prepared in water containing 0, 150, or 300 mM KCl, respectively. In accordance with previously published results high concentrations of salt result in an enhancement of the transition enthalpy of the sub-main transition [9,10]. As shown in Fig. 1B no volume change can be detected at the sub-main transition for vesicles made in pure water. However, at 300 mM KCl a significant volume increase at the sub-main transition is observed. This observation further substantiates the conclusion that the addition of salt makes the sub-main transition more pronounced.

Fig. 4A shows the heat capacity, C_p, obtained from Monte Carlo computer simulations of a $DC_{18}PC$ lipid bilayer where 15% of the lipids are exposed to a locally reduced lateral pressure corresponding to $\Pi = 27.5$ dyne/cm. The figure also shows

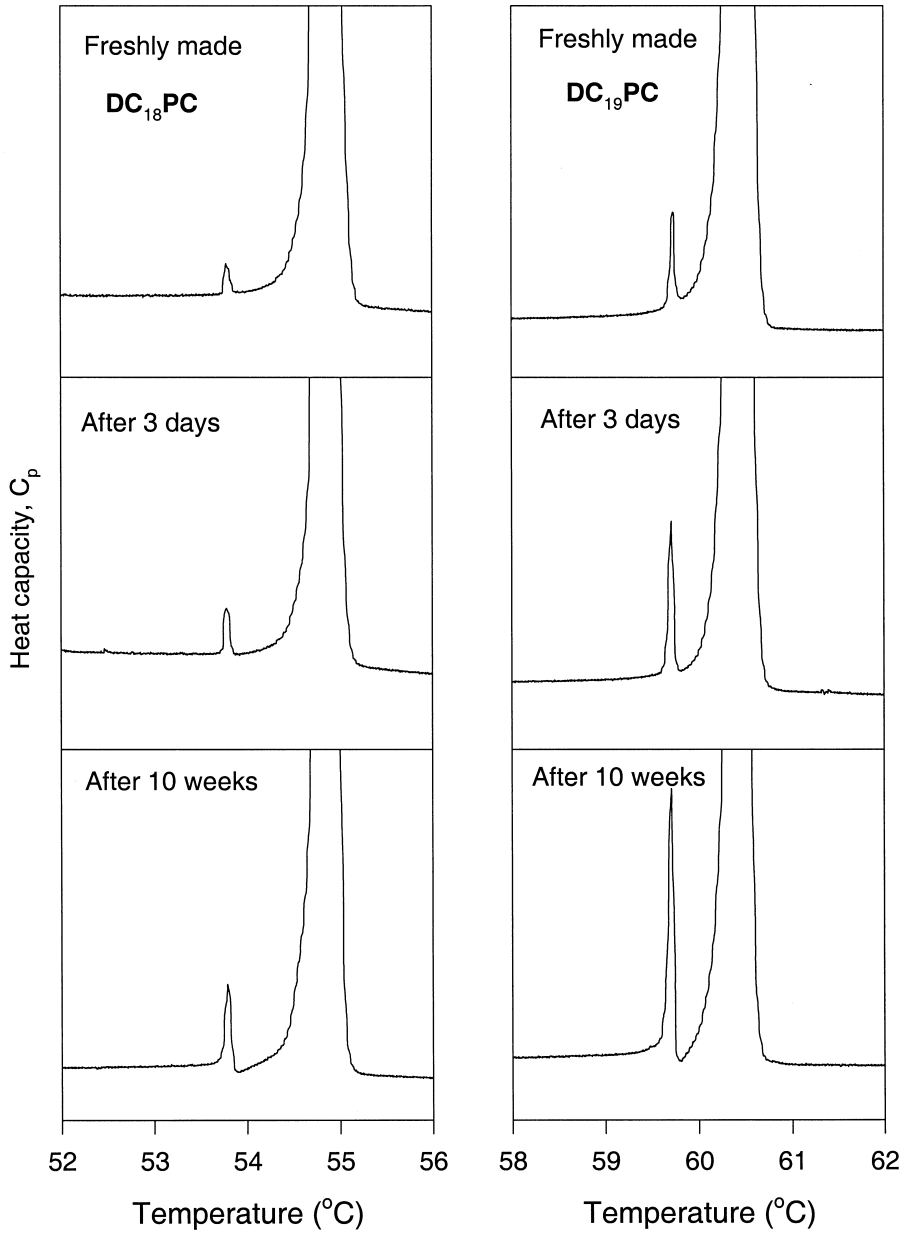

Fig. 2. Heat capacity, C_p, obtained by differential scanning calorimetry at a scan rate of 4°C/h for multilamellar vesicles composed of $DC_{18}PC$ (left) or $DC_{19}PC$ (right). The heat capacity curves have been obtained on vesicles that are freshly made in pure water or have been stored for either 3 days or 10 weeks.

simulation results for a $DC_{18}PC$ system that is characterized by a global and uniform lateral pressure of $\Pi = 30$ dyne/cm. The single main peak in the C_p curve appearing at $T = 54.7$°C when the global lateral pressure is $\Pi = 30$ dyne/cm corresponds to the well-known chain melting main transition taking place in saturated phospholipid bilayers [12]. However, the appearance of a small second peak at $T = 53.6$°C on the low-temperature side of the main endotherm reflects a disordering of those lipid acyl chains that are characterized by a locally reduced lateral pressure. Concomitantly, a small reduction of the heat capacity peak height and the melting enthalpy associated with the major endotherm at $T = 54.7$°C takes place.

The lateral bilayer structure as obtained from the

computer simulation calculations at a temperature below the main transition is shown in Fig. 4B. The snapshot, which has been obtained at 53.8°C on the high-temperature side of the minor endotherm (marked by the arrow in Fig. 4A), visualizes the effect of having a locally decreased lateral pressure in a small part of the lipid bilayer. This is manifested as a lateral bilayer structure composed of coexisting ordered and disordered $DC_{18}PC$ lipids, e.g., gel and fluid phase coexistence. It is noted that the lateral bilayer structure within the coexisting fluid and gel phases is characterized by fluctuating gel and fluid domains, respectively [12]. In the simulation study we have made no particular attempt to fit the model to the experimental data. If a larger fraction of the lipid bilayer is exposed to a reduced lateral pressure, an increase of the small endotherm would be observed and vice versa. However, a striking qualitative

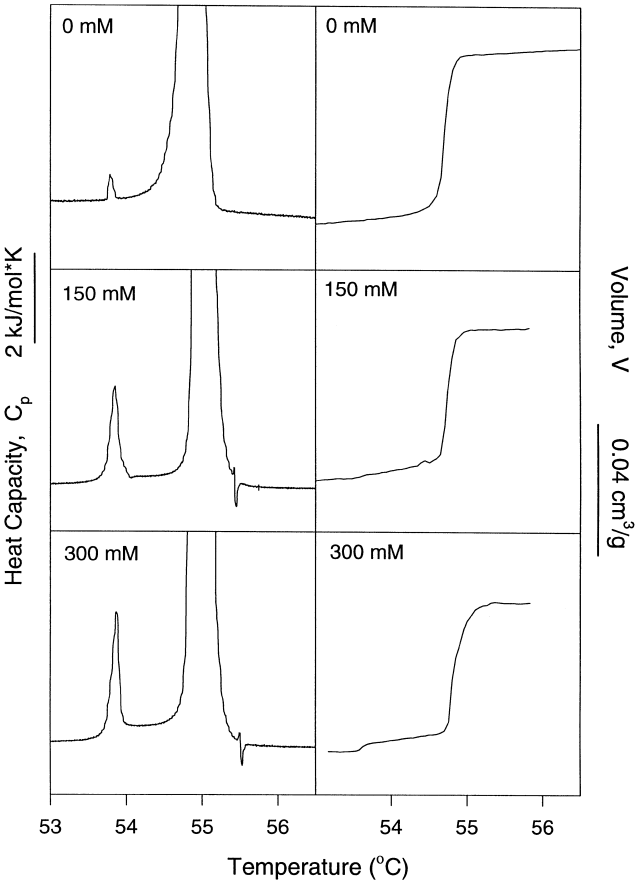

Fig. 3. Heat capacity, C_p (left), and specific volume, V (right), of $DC_{18}PC$ multilamellar vesicles in aqueous solution containing 0, 150 mM, and 300 mM KCl.

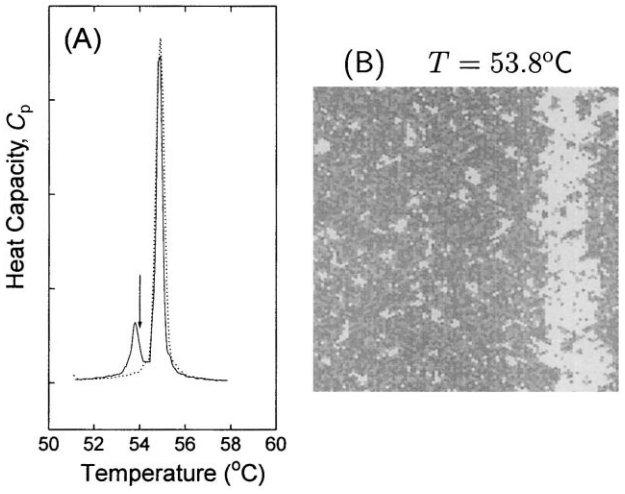

Fig. 4. (A) Heat capacity, C_p, as a function of temperature obtained from computer simulations on $DC_{18}PC$ lipid bilayers characterized by a global lateral pressure of $\Pi = 30$ dyne/cm (dotted line) and a reduced lateral pressure of $\Pi = 27.5$ dyne/cm (heavy line) in 15% of the lipid bilayer. (B) Snapshot of the lateral $DC_{18}PC$ lipid bilayer structure obtained on the high temperature side of the small endotherm as marked by the arrow in A. Ordered and disordered lipid acyl chains in the lipid bilayer are marked as dark grey and light grey regions, respectively.

similarity is observed by comparison of the computer simulation results of the heat capacity in Fig. 4B with the experimentally obtained results in Figs. 2 and 3. The combined experimental and simulation results suggest that the overall mechanism involved in the sub-main transition is related to a locally decreased lateral bilayer pressure and an associated effect on microscopic molecular properties such as the conformational acyl chain states which in turn might lead to phase coexistence [18]. In accordance with this earlier X-ray results have demonstrated that the structural changes involved in the sub-main transition might involve a partly disordering of the lipid acyl chains in certain regions of the lipid bilayer [9].

It has been argued that the second endotherm (the sub-main transition) involves an osmotic stress created by solutes in the aqueous phase [10]. Our results, however, clearly reveal that the osmotic stress hypothesis does not provide a sufficient explanation of the mechanisms involved in the sub-main transition, e.g., the possibility of creating a concentration gradient across the lipid bilayer is excluded in vesicles made in pure water (cf. Figs. 1–3). Moreover, our results demonstrate that the intensity of the sub-

main transition is enhanced when the vesicles are allowed to equilibrate at low temperatures for several weeks. Interestingly, a recent study has shown that increasing concentrations of ions were able to induce phase coexistence and a change in the lipid bilayer hydration [18]. Furthermore, it has been proposed that the existence of ordered and disordered regions in the ripple phase is closely related to a locally increased curvature caused by the bilayer rippling [16,17]. Our combined experimental and simulation studies suggest that an intimate relationship exists between a local decrease of the lateral pressure in part of the bilayer and the associated effects on the conformational states of the lipid acyl chains. However, we are unable to provide an explanation of the detailed and underlying molecular mechanisms involved in the sub-main transition. A change in the lipid bilayer hydration level in combination with a curvature stress resulting in phase coexistence may be of relevance although other effects also have to be considered in future studies.

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