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Rapid report

Calorimetric detection of a sub-main transition in long-chain phosphatidylcholine lipid bilayers

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Abstract

The existence of a sub-main transition in multilamellar bilayers composed of long-chain saturated diacyl phosphatidylcholine ($DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$, and $DC_{20}PC$) is reported for the first time using high-sensitivity differential scanning calorimetry. The highly cooperative sub-main transition which takes place over a narrow temperature range positioned between the well-known pre-transition and main-transition is characterized by a heat capacity curve with a half-height width of $\Delta T_{1/2} \simeq 0.15$ C° and an enthalpy change, ΔH, which is a few percent of the transition enthalpy for the main-transition of the lipid bilayer.

Keywords: Lipid bilayer; Phospholipid; Phase transition; Sub-main transition; DSC

Saturated phospholipid bilayers can undergo a series of thermotropic phase transitions which are considered to be of relevance for biological membranes [1,2]. The most extensively studied transition is the chain melting gel-fluid main-transition which takes the bilayer from a low temperature gel-phase dominated by ordered acyl chain conformations to a high temperature fluid-phase characterized by highly disordered acyl chain conformations [3]. In addition to the main-transition, saturated diacyl phosphatidylcholine lipid bilayers are known to undergo phase transitions like e.g. the sub-transition and the pre-transition. The sub-transition is a low-temperature chain packing transition involving crystalline phases [4] whereas the pre-transition takes place from several to a few degrees below the main-transition depending on the acyl chain length of the lipids constituting the bilayer [5,6]. The pre-transition involves a two-dimensional reorganization of the lipid bilayer structure and is generally ascribed to an interplay between the packing constrains of the bulky phosphatidylcholine headgroups and the long hydrophobic acyl chains. This leads to a transition with a changed orientation of the acyl chains with respect to the bilayer normal and a two-dimensional structure with long-range order known as the rippled phase [4]. The pre-transition and the main-transition have been intensively studied both in one-component and two-component saturated phospholipid bilayers incorporated with various molecular compounds like cholesterol, peptides or drugs [3]. In particular, many of the studies have focused on $DC_{16}PC$-lipid bilayers as model membranes for both experimental and theoretical investigations of how various molecular compounds are incorporated in or interact with the lipid bilayer with a subsequent influence on the transitional membrane properties of relevance for active and passive membrane processes [7,8].

In this paper we present high-sensitivity differential scanning calorimetric results which show for the first time that a highly cooperative sub-main transition exists for a series of saturated diacyl phospholipid bilayers of different acyl chain length. The heat capacity measurements demonstrate that a low-enthalpy phase transition which is positioned between the pre-transition and the main-transition exists in one-component lipid bilayers composed of $DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$, and $DC_{20}PC$.

Methods and materials. The lipids dihexadecanoic phosphatidylcholine ($DC_{16}PC$), diheptadecanoic phosphatidylcholine ($DC_{17}PC$), dioctadecanoic phosphatidylcholine ($DC_{18}PC$), dinonadecanoic phosphatidylcholine

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

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($DC_{19}PC$), dieicosanoic phosphatidylcholine ($DC_{20}PC$), and didocosanoic phosphatidylcholine ($DC_{22}PC$) were purchased from Avanti Polar Lipids (Birmingham, AL, USA) and used without further purification. The purity of the lipids was checked using a Waters Millenium 2010 (Milford, MA, USA) high performance liquid chromatography system mounted with an evaporative ACS (Cheshire, UK) mass detector, a 5 μm Phenomenex (Torrance, CA, USA) diol spherical column and a mixture of chloroform/methanol/water (7.3:2.3:0.3, v/v) as mobile phase. Only a single peak was observed in the HPLC chromatograms confirming the purity of the lipids being greater than 99%. The multilamellar vesicles were made by dispersion of an appropriate weighed amount of the lipid in a 50 mM KCl and 1 mM NaN_3 solution. The lipid suspension was allowed to stand for at least one hour at a temperature ten degrees above the main-transition temperature, T_m, of the lipid species in question. During this period the lipid suspension was vortexed several times. Differential scanning calorimetry was performed using a MicroCal MC-2 (Northhampton, MA, USA) ultra-sensitive power compensating calorimeter equiped with a nanovoltmeter to improve sensitivity. 1.2 ml of 5 mM and 0.8 mM lipid samples were scanned at different scan rates of which the lowest was 4 C°/h and the fastest 60 C°/h. An equilibration period of 50 min was used before each scan. An appropriate baseline was subtracted from the excess heat capacity curves and no correction of the curves were made for the fast time response ($\tau \approx 10$ s) of the calorimeter.

Results and discussion. Fig. 1A and 1B show differential scanning calorimetric results for pure one-component multilamellar vesicles composed of $DC_{16}PC$, $DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$, $DC_{20}PC$ and $DC_{22}PC$ lipids as obtained at a scan rate of 13 C°/h. The curves presented in Fig. 1A and 1B show one out of three consecutive scans performed for each lipid species. The heat capacity curves in Fig. 1B clearly demonstrate the existence of a low-enthalpy sub-main transition for lipid bilayers composed of phospholipids having from 17 to 20 carbons atoms in the saturated acyl chains. The low-enthalpy sub-main transition which is characterized by a heat capacity curve with a half-height width of $\Delta T_{1/2} \simeq 0.15$ C° for e.g. $DC_{18}PC$-lipid vesicles indicates a high degree of cooperativity and a first-order nature of the transition. The small peak of the sub-main transition which is positioned between the well-known pre-transition and main-transition moves closer towards the main-transition as the chain length increases from 18 to 20 carbon atoms and it seems as if the sub-main transition disappears when the chain length becomes shorter than 17 carbon atoms and longer than 20 carbon atoms. Whether the disappearance of the sub-main transition for $DC_{16}PC$ lipids is due to a transition enthalpy which becomes undetectable for lipid bilayers composed of lipids having less than 17 carbon atoms in the acyl chains is at present unresolved. The apparent broadening of the single peak transition observed for the $DC_{22}PC$ multilamellar vesicles might reflect that both the pre-transition, the main-transition and possible the sub-main transition take place at temperatures which are very close leading to a superposition of the transition enthalpies. The heat capacity curves in Fig. 2A and 2B of two successive upscans of $DC_{18}PC$-lipid vesicles obtained at scan rates of 4 C°/h and 60 C°/h indicate that the kinetics of the sub-main transition is faster than the slow kinetics of the pre-transition [9]. Increasing scan rate from 4 C°/h to 60 C°/h gives rise to a peak position of the pre-transition

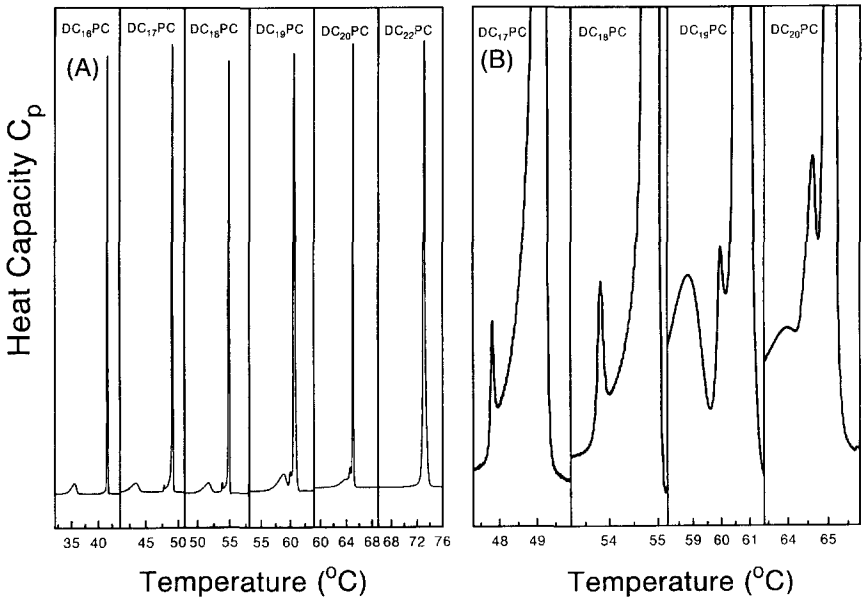

Fig. 1. (A) Heat capacity curves as obtained by DSC at a scan rate of 13 C°/h for multilamellar bilayers composed of $DC_{16}PC$, $DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$, $DC_{20}PC$, and $DC_{22}PC$. (B) Magnification of the heat capacity curves shown in (A) for multilamellar bilayers composed of $DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$, and $DC_{20}PC$.

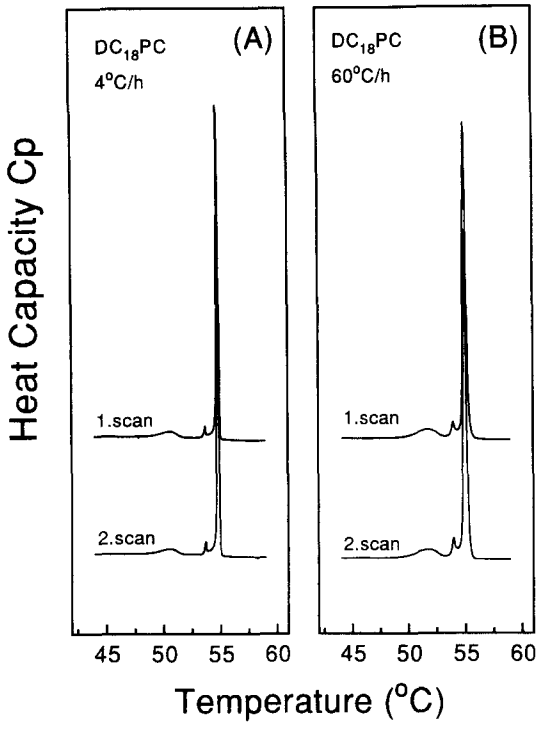

Fig. 2. Evolution of heat capacity curves during two successive upscans for multilamellar vesicles composed of $DC_{18}PC$ lipids as obtained by DSC at a scan rate of 4 C°/h (A) and a scan rate of 60 C°/h (B).

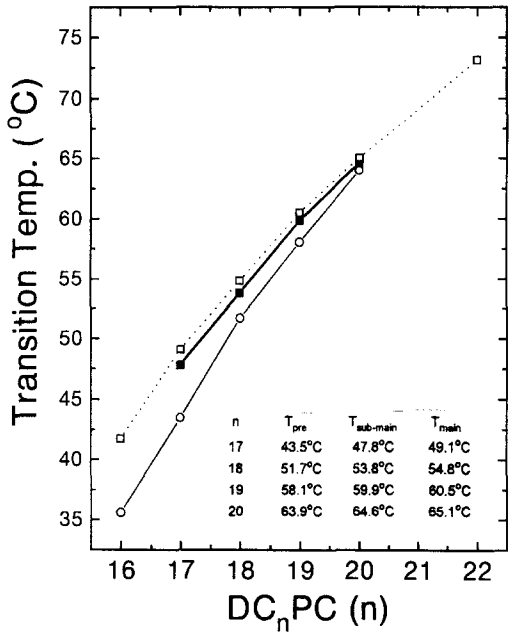

Fig. 3. Transition temperatures, T_m, determined from the peak position of the heat capacity curves as obtained by DSC at a scan rate of 13 C°/h for the pre-transition (thin line), the sub-main transition (heavy line), and the main-transition (dotted line) as a function of the acyl chain length (n) of DC_nPC multilamellar vesicles. The inserted table shows the transition temperatures for the pre-transition, the sub-main transition and the main transition.

which moves closer to the peak position of the main-transition, whereas the peak position of the sub-main transition displays very little scan rate dependency. Analogous differential scanning calorimetric results as shown in Fig. 2A and 2B demonstrating both the fast kinetics and the reversibility of the sub-main transition have been obtained both in the upscan mode and the downscan mode for multilamellar vesicles composed of longer acyl chain lipids like e.g. $DC_{19}PC$ (data not shown). Moreover similar results as shown in Fig. 2 have been obtained using high-grade lipids purchased from Sigma (St. Louis, MO, USA) suspended in plain water (Jørgensen, unpublised results). Fig. 3 displays the transition temperatures for the pre-transition, the sub-main transition and the main-transition as a function of lipid acyl chain length, n determined from the peak position of the heat capacity curves as obtained by DSC. The figure clearly shows that the sub-main transition moves closer to the main-transition as the chain length increases and possible coalesces with the main-transition when the chain length increases from 20 to 22 carbon atoms. In Fig. 4 is shown the transition enthalpy, ΔH, for the sub-main transition as a function of acyl chain length, n. The figure reveals that the transition enthalpy for the sub-main transition increases as the chain length becomes longer. An accurate determination of the enthalpy of the sub-main transition obviously involves a baseline subtraction which becomes troublesome when the sub-main transition takes place at a temperature which is very close to the main-transition temperature as the case for for e.g. $DC_{19}PC$ and $DC_{20}PC$ vesicles.

The molecular mechanisms underlying the observed sub-main transition is at present unknown. It remains unclear whether the observed transition can be related to a

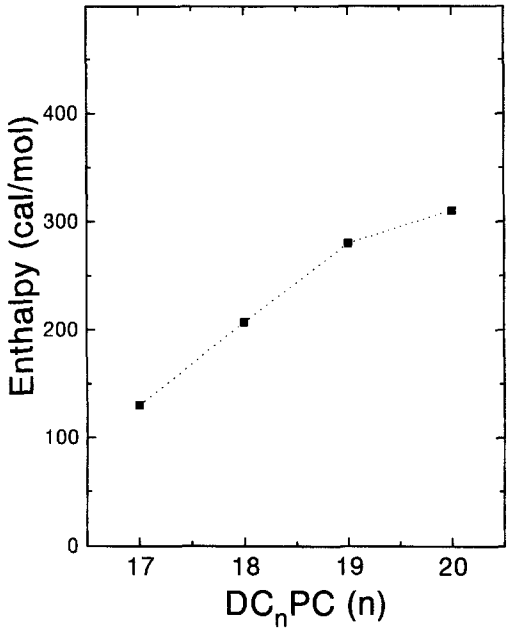

Fig. 4. Transition enthalpy, ΔH, as determined from the heat capacity curves obtained by DSC at a scan rate of 13 C°/h for the sub-main transition as a function of the acyl chain length (n) of DC_nPC multilamellar vesicles. The transition enthalpies for $DC_{17}PC$, $DC_{18}PC$, $DC_{19}PC$ and $DC_{20}PC$ are 130, 205, 280 and 310 cal/mol, respectively.

two-dimensional micro-domain crystalization phenomena related to the headgroup regions. The possibility exists that the sub-main transition is a decoupling transition involving the positional and configurational order of the lipid chains which leads to an intermediate phase characterized by ordered acyl chains but a lack of two-dimensional order as suggested by Zuckermann and Mouritsen [10]. Obviously, further experimental work is required in order to understand the nature of the sub-main transition and to determine whether the transition found in the multilamellar vesicles also is present in unilamellar bilayer systems. Results obtained for unilamellar vesicles might help clarifying to which extent the sub-main transition is related to a two-dimensional rearrangement and possible coupling between the headgroup regions of the stack of bilayers in the multilamellar vesicles. The influence and importance of the sub-main transition in relation to experimental investigations of lipid bilayer systems incorporated with various molecular compounds remains unclear, although a careful reinspection of previous differential scanning calorimetric results obtained in our laboratory has shown that the sub-main transition is highly sensitive to the presence of membrane interacting compounds like lindane [11]. To gain further insight into the molecular mechanisms of the highly cooperative sub-main transition and the nature of the associated thermodynamic phases further experimental investigations are required which can provide information about the structural organization of the two-dimensional membrane structure, e.g. atomic force microscopy, X-ray or neutron scattering experiments. The results of the present work, however, indicates that it is of absolute importance that experimental studies related to a better characterization of the sub-main transition has an optimal temperature control and resolution due to the position and narrow temperature span (half height width, $\Delta T_{1/2} \simeq 0.15$ C°) of the so far over-looked sub-main transition. In particular a better understanding of the molecular phenomena underlying the sub-main transition is required due to the widespread use of saturated diacyl phospholipid bilayers as model systems to gain insight into the structural transitional properties of relevance for active and passive membrane processes.

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