ELSEVIER

Biochimica et Biophysica Acta 1283 (1996) 170–176

BBA Biochimica et Biophysica Acta

Model of a sub-main transition in phospholipid bilayers

Morten Nielsen [a], Ling Miao [a], John H. Ipsen [b], Kent Jørgensen [b], Martin J. Zuckermann [a,*], Ole G. Mouritsen [b]

[a] *Centre for the Physics of Materials, Department of Physics, McGill University, 3600 University Street, H3A 2T8 Montreal, Canada*
[b] *Department of Physical Chemistry, The Technical University of Denmark, Lyngby, Denmark*

Received 3 January 1996; revised 26 April 1996; accepted 3 May 1996

Abstract

A recently discovered submain phase transition in multi-lamellar bilayers of long-chain saturated diacyl phosphatidylcholines (Jørgensen, K. (1995) Biochim. Biophys. Acta 1240, 111–114) is discussed in terms of a theoretical molecular interaction model using computer simulation techniques. The model interprets the transition to be due to a decoupling of the acyl-chain melting from the melting of the pseudo-two-dimensional crystalline lattice of the $P_{\beta'}$ phase. A two-stage melting process is predicted by the calculations suggesting that the sub-main transition involves a lattice melting whereas the acyl-chain melting takes place at a higher temperature at the main transition. The calculated heat contents of the two transitions as well as the chain-length dependence compare favorably with experimental data for multi-lamellar phosphatidylcholine lipid bilayers.

Keywords: Lipid bilayer; Phospholipid; Submain phase transition; Theoretical model; Chain melting; Lattice melting; Calorimetry

1. Introduction

The study of phase equilibria in lipid-bilayer systems continues to reveal unexpected results even for the well-studied family of diacyl saturated, phosphatidylcholines. New transitions involving sub-gel phases were discovered in the eighties [1–3], transitions that hitherto had not been observed mainly due to extremely slow kinetic effects. Another transition in the fluid phase involving changes in lipid morphology was reported in 1989 by Gershfeld [4]. This transition was previously neglected probably because no transition was expected to take place in fluid lipid bilayers. Most recently, Jørgensen [5] reported a new sub-main phase transition in long-chain phospholipid bilayers of DCnPC with $17 \leq n \leq 20$. The sub-main transition, which is the most cooperative and sharpest phase transition so far observed in lipid bilayers, was previously overlooked because it has a calorimetric width of only 0.15°C and it occurs within one degree of the main phase transition.

For several of the minor lipid-bilayer phase transitions the details of the mechanism which triggers the transition remain elusive. In the case of the sub-main transition, only bulk thermodynamic data is currently available whereas structural studies are still waiting to be performed. The mechanism of this transition therefore remains fully unknown. The sub-main transition is only observed for long-chain lipids in the homologous series DCnPC, with $17 \leq n \leq 20$, and is characterized by a rather small transition enthalpy, ΔH_{s-m}. Both ΔH_{s-m} and the transition temperature, T_{s-m}, display a systematic chain-length dependence [5]. These results imply that the transition mechanism is rather subtle and probably does not involve the polar-head region of the lipids. With the polar-head-group variables of the lipid molecules neglected, there are two basic mechanical variables in terms of which the lipid-bilayer aggregate can undergo condensing transitions. One is the acyl-chain conformational variable, that describes the degree of internal acyl-chain molecular order. The other is the translational variable, that describes the lateral position of the molecule in the plane of the bilayer. It is usually assumed that the main transition in lipid bilayers takes place by

Abbreviations: DCnPC, saturated diacyl phosphatidylcholine with n carbon atoms in each acyl chain; DSC, differential scanning calorimetry; DSPC, distearoyl phosphatidylcholine ($DC_{18}PC$).

* Corresponding author. Fax: +1 (514) 3986526; e-mail: martin@physics.mcgill.ca.

PII S0005-2736(96)00086-7

condensation in terms of both of these variables at the same time. In the present paper we propose that the observed sub-main transition is a manifestation of a decoupling of the two transitions in the translational and conformational variables, respectively.

Our arguments are presented and rationalized in terms of a molecular interaction model whose thermodynamic and transitional properties are calculated by computer-simulation techniques. The model provides a convenient framework for a discussion of the sub-main transition and its dependence on various system parameters, such as acyl-chain length. The model and its predictions are useful for focussing further experimental investigations of the sub-main phase transition.

In Section 2 we describe briefly the phenomenology of acyl-chain melting and lattice melting in lipid bilayers using the concept of translational and conformational variables for lipid molecules. A molecular interaction model describing the coupling between the two sets of variables is advanced in Section 3, together with a description of the computer-simulation techniques used to calculate the phase diagram and the specific heat of this model. The proposed model should be regarded as a minimal model in the sense that it is the simplest possible microscopic model which contains the necessary physics to study the coupling between the two types of variables. Section 4 contains the results of the theoretical calculations. Finally, Section 5 is devoted to a discussion of the results obtained and a comparison with calorimetric experiments for the sub-main phase transition together with suggestions for theoretical refinements and further experimental work.

2. Phenomenology of chain melting and lattice melting

The main phase transition in DCnPC lipid bilayers proceeds from a solid (gel) phase, the $P_{\beta'}$-phase, to a liquid (fluid) phase, the L_α-phase [6]. We will refer to the $P_{\beta'}$-phase, also known as the ripple phase, as a solid-ordered (**so**) phase as it is a crystalline solid associated with translational order as well as a high degree of conformational acyl-chain order. The $P_{\beta'}$-phase is therefore ordered in terms of both translational and conformational variables. The L_α-phase is a liquid phase characterized by lateral disorder and a high degree of acyl-chain disorder; hence it is disordered in terms of both types of variables and is thus termed a liquid-disordered (**ld**) phase. These phase labels were originally introduced in connection with the description of the phosphatidylcholine-cholesterol phase diagram [7,8] which at high cholesterol concentrations exhibits a cholesterol-induced liquid-ordered phase (**lo**) characterized as a liquid with translational disorder, but in which the acyl chains have a high degree of conformational order reminiscent of the gel phase.

In principle there can be two distinct types of melting processes in a one-component lipid bilayer, corresponding to a change in the translational variables, $\mathbf{s} \rightarrow \mathbf{l}$, and a change in the conformational variables, $\mathbf{o} \rightarrow \mathbf{d}$, respectively. It is usually assumed that at the main transition the two melting processes are strongly coupled and take place simultaneously, i.e. $\mathbf{so} \rightarrow \mathbf{ld}$, without the occurrence of an intermediate phase, **lo** [9]. There is, however, no a priori reason to expect that this should in general be the case. For certain lipid monolayers it has been suggested that the two transitions may be decoupled [10,11]. In lipid bilayers, the induction of a **lo** phase by cholesterol has been explained in terms of the ability of cholesterol to decouple the two transitions [7]. The two types of order-disorder processes that can take place in lipid bilayers can be studied directly by spectroscopic or thermodynamic measurements (the $\mathbf{o} \rightarrow \mathbf{d}$ transition) and high angle scattering techniques (the $\mathbf{s} \rightarrow \mathbf{l}$ transition), respectively [12].

3. Model and calculational techniques

In order to understand the relevant physics involved in the sub-main phase transition, we propose a minimal theoretical model which is able to describe the basic thermodynamic phase behavior of the lipid bilayer system. Full details of this model will be given elsewhere (Nielsen, M., Miao, L., Ipsen, J.H., Mouritsen, O.G., and Zuckermann, M.J., unpublished). A related minimal model for the decoupling of the $\mathbf{s} \rightarrow \mathbf{l}$ and $\mathbf{o} \rightarrow \mathbf{d}$ transitions in the case of lipid monolayers was discussed in Ref. [13]. The present model is an extension of a two state lattice model proposed by Doniach [14]. In this original model the phase behavior of the conformational variable of the lipid acyl-chains is modelled via two chain states: One state is associated with the ordered state of the acyl-chain and has zero internal conformational energy and is non-degenerate; the other state is associated with the disordered state of the chain and is highly degenerate (representing the large number of possible chain conformations) and has a large internal conformational energy. In the lattice model, each site of a triangular lattice is occupied by one acyl-chain. The main difference between our model and the lattice model of Doniach is that each chain in our model is allowed to have a varying number of nearest neighbors and varying distances from its neighbors in addition to the two distinct conformational states. Furthermore, the chains are allowed to diffuse through the whole system, this being another manifestation of the translational invariance of the system. The internal and the translational variables are then coupled through intermolecular interactions.

In our two-dimensional model the lipid molecules (or acyl chains) diffuse on a two-dimensional surface under the constraint of a lateral pressure, P. Each chain has a hard core corresponding to a hard disc of radius R_0. In the following we shall for convenience refer to these hard discs as the chains. The attractive intermolecular interaction between any two chains in the ordered state is approx-

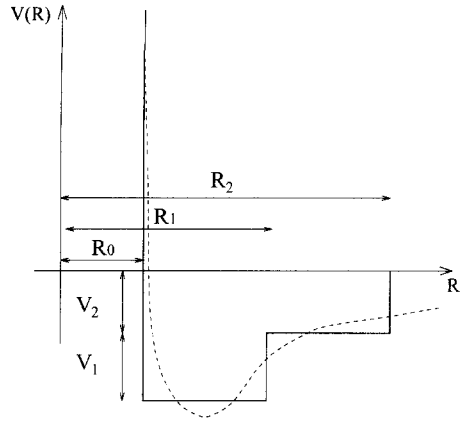

Fig. 1. Schematic illustration of the model potential, $V(R)$, consisting of a sum of a hard-core potential and two square-well potentials. The hardcore radius is R_0, and the range and strength of the square-well potentials are (R_1, R_2) and (V_1,V_2), respectively. The dashed line illustrates a Lennard-Jones-type potential to which the model potential is an approximation.

imated by the sum of two square-well potentials of different range (R_1, R_2; $R_1 < R_2$) and different strength (V_1, V_2). The sum of the hard-core potential and the two square-well potentials constitutes an approximation to a standard intermolecular potential of the Lennard-Jones type. The model potential is illustrated in Fig. 1. On the scale of these interaction parameters, the effective interaction between any two chains is taken to be zero if either one or both of the chains are in the disordered state. This is clearly an approximation. The model is now naturally described in terms of the reduced variable V_1/V_2. The square-well potential described by R_1 and V_1 controls the minimum of the potential and hence the lattice parameter of the crystalline solid phase. This model potential is a minimal potential in the sense that it contains only the most essential physics required to model a system with translational and internal-state variables interacting with pairwise interactions. The hard core is required for the system not to collapse at low temperatures and high pressures, the deep well at short range is needed for producing the crystalline phases, and the tail of the potential extending beyond the short range permits a possible decoupling of the phase transition in the translational variables from that in the conformational chain variables.

In the same way as for the Doniach model [14], our model as a statistical mechanical model is formally related to an Ising model in an external temperature-dependent field. The thermodynamic behavior of the model is thus determined by the direction and strength of this external field. At low temperatures, the effective field is non-zero and aligns the chains in the ordered state. Moreover, the thermal density fluctuations are too weak to excite the chains out of the square-well potential and the system is frozen in a crystalline triangularly ordered structure. The low-temperature phase is thus the **so** phase, where both the chain ordering and the lateral ordering are present. As the temperature is increased, the effective field progressively loses its strength in enforcing the chain-ordered state, becomes zero at a temperature T_m and then switches its direction, preferring the chain-disordered state at temperatures higher than T_m. Correspondingly, the ordering of the chains undergoes a discontinuous change at T_m. Since the chains interact only very weakly when they are in the disordered state, thermal fluctuations in density are always strong enough to destroy the lateral ordering of the system when there is no chain ordering; the high-temperature phase is the **ld** phase. The transition from the **so** phase to the **ld** phase can proceed via two scenarios, depending on the strength of the deeper well in the interaction potential (Fig. 1) relative to thermal energy. If thermal fluctuations below T_m are too weak to excite the system out of the deeper well of the potential, the lateral ordering of the system persists until it is destroyed by the chain melting. The transition is therefore a first-order transition directly from **so** phase to **ld** phase without the intervention of the intermediate phase, **lo**. If, however, the thermal fluctuations in the density are strong enough to overcome the confinement of the deeper well at some temperature, T_{s-m}, below T_m, the system then first makes a transition at this temperature from the chain-ordered/crystalline-ordered **so** phase to an intermediate, chain-ordered/crystalline-disordered **lo** phase, and undergoes a subsequent transition from **lo** to **ld** at the higher temperature T_m. The overall phase behavior of the system is then given by the sequence **so** → **lo** → **ld**, as a result of the decoupling between the chain-conformational degrees of freedom and the translational degrees of freedom. It is this two-stage melting process that we will associate with the recently discovered sub-main phase transition in multi-lamellar bilayers of long-chain phospholids, DCnPC [5].

The phase behavior of the model was investigated using the Metropolis Monte Carlo algorithm [15]. The algorithm that treats the translational variables of the lipid molecules, is a two-dimensional version of the dynamic-triangulation algorithm used for modelling fluctuating fluid membranes in three dimensions [16]. The simulations were carried out on a finite system of N chains subject to periodic boundary conditions and were performed in the NPT ensemble (i.e. constant N, constant P, and constant T). The system was initiated in a triangular crystalline configuration with a high-temperature disordered chain conformation and equilibrated using the Metropolis Monte Carlo algorithm resulting in a high-temperature equilibrium state characterized by both translational disorder and acyl-chain disorder. The high-temperature state was then cooled to a low-temperature state for different values of the relative depth of the two square-well potentials. In the simulations we calculated the total enthalpy and the thermally-averaged total area of the system. Furthermore the chain order parameter and the lateral structure (through the structure factor) were investigated (Nielsen, M., Miao, L., Ipsen, J.H., Mouritsen, O.G., and Zuckermann, M.J., unpublished) and used

to characterize the different phases. Finally, we used our simulation data to calculate the isobaric specific heat, the area compressibility and the susceptibility of the chain order parameter using expressions derived from the fluctuation-dissipation theorem [17], and the singularities in these thermodynamic response functions were then used in resolving the location of the various phase boundaries. In this way we were able, for a particular choice of the model parameters, to determine the complete phase behavior of the model as a function of V_1/V_2.

4. Simulation results

The calculated phase diagram for our model is displayed in Fig. 2 in terms of reduced temperature, k_BT/V_2, and V_1/V_2. The two phase boundaries representing the **ld–lo** and **ld–so** transitions were determined from the position of peaks in the specific-heat curves. The simulations were performed using a lateral pressure of $P = 5.0V_2/R_0^2$ for $N = 256$ chains. It is evident that the two phase transitions are decoupled for values of V_1/V_2 smaller than a specific (triple-point) value and become coupled at the triple point. For values of V_1/V_2 above the triple-point value, the two transitions are strongly coupled and the transitions in the translational (**s** → **l**) and chain (**o** → **d**) variables take place simultaneously. In insets to the phase diagram in Fig. 2 we show snapshots of micro-configurations illustrating the lateral structure of the three different phases obtained from the simulations.

The temperature difference between the two decoupled phase transitions, **so** → **lo** and **lo** → **ld**, can be varied in two ways. One way is to change either the relative depth of the two square-well potentials or the magnitude of the lateral pressure, P, and thus adjust the position of the lattice melting transition. The other way is to adjust the position of the chain-melting transition point by varying the model parameters E_β, D_β and V_2, where E_β is the excitation energy associated with the conformational change from the ordered to the disordered state, D_β is the degeneracy of the disordered state, and V_2 is the strength of the longer range chain–chain interaction. The chain-melting transition temperature, T_m, is found to be related to the model parameters as [1] [14]

$$T_m \simeq \frac{E_\beta + 12V_2 + P\Delta A}{k_B \ln D_\beta}, \qquad (1)$$

where ΔA is the area change per chain at the main transition. In the relevant range of parameters, the pressure term, $P\Delta A$, in Eq. (1) plays a negligible role and can therefore be omitted.

The dependence of the lattice-melting transition on model parameters V_1 and V_2, is given in the phase diagram (Fig. 2), and may be explicitly expressed as $\alpha(V_1/V_2)V_2/k_B$, where $\alpha(V_1/V_2)$, a function of V_1/V_2, is obtained numerically. This information, along with Eq. (1), allows us to assess the general chain-length dependence of the phase behavior of the model, provided that the chain-length dependence of all the model parameters is known. The dependence on chain length n of the two parameters characterizing chain conformation, E_β and $\ln D_\beta$, has been discussed in other theoretical model work [18,19]. In general it is expected that both parameters are linear in n. The precise chain-length dependence of the interaction parameters, V_1 and V_2, is harder to obtain; and we only make an estimate with the aid of experimental data and Eq. (1). Since most of the latent heat in the main transition is due to the melting of the lipid chains, we can from experimental observation determine $k_BT_m \ln D_\beta \simeq \Delta H_m$ [2], which increases with chain length. Substituting this into Eq. (1), we then find that V_2 also increases as chain length increases [3]. Finally, we assume that, to a first approximation, the reduced variable, V_1/V_2, has negligible chain-length dependence. Hence, the lattice-melting temperature assumes its chain-length dependence through $V_2(n)$, as it is described by $\alpha(V_1/V_2)V_2(n)/k_B$, where $\alpha(V_1/V_2)$ is essentially a constant with respect to changes in the chain length. By comparing this n dependence with that of the chain-melting temperature, which is mainly given by $12V_2(n)/(k_B \ln D_\beta)$ (from Eq. (1)), it may be concluded that the lattice-melting temperature increases with the chain length at a faster rate than the chain-melting temperature. In other words, the difference between the two transition temperatures diminishes as the acyl chain length increases.

The specific heat is of direct relevance to calorimetric data on the phase behavior of lipid bilayers. The simulation data in Fig. 3 gives the theoretical specific-heat curve for a single temperature scan at a value of $V_1/V_2 = 0.5$. The scan clearly shows two distinct phase transitions as the system is cooled down from a high temperature state. At a temperature, $T_m = 2.55V_2/k_B$, a very sharp transition representing the main phase transition takes the lipid system from the liquid-disordered or **ld** phase to an **lo** phase

[1] The chain-melting temperature is always obtained from simulation data. The numerical value agrees very well with this expression, which can easily be derived from the mean-field theory of the model. Here, we use this mean-field result to illustrate explicitly the dependence of T_m on various model parameters.

[2] In order to make a direct comparison between the results from the model calculation and experimental observation we shall refer to the transition entropy since this quantity has no explicit model parameter dependency. For $DC_{18}PC$ multilamellar vesicles the chain melting transition temperature T_m is 54.8°C and the melting enthalpy $\Delta H_m = 10.9$ kcal/mol (cf. data in Fig. 4). This corresponds to a transition entropy per molecule of $S_m = \Delta H_m / T_m = k_B \ln D_\beta = 16.7\ k_B$.

[3] This dependence is not expressed analytically and will not be explicitly given in the text. In fact, it is already sufficient for our general argument to know that V_2 increases monotonically with n.

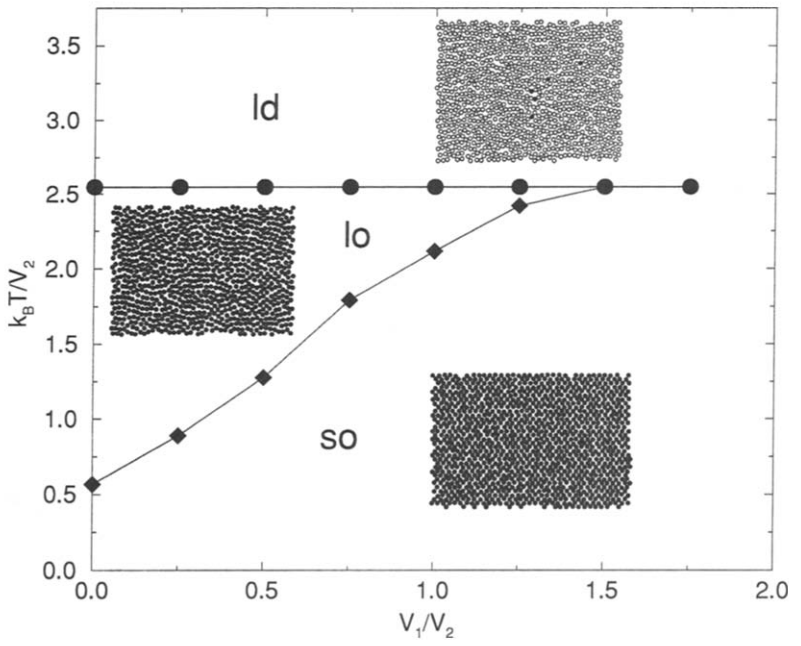

Fig. 2. Theoretical phase diagram and snapshots of typical micro-configurations for the three different phases labelled **so** (solid-ordered), **ld** (liquid-disordered), and **lo** (liquid-ordered). Chains in the disordered state are plotted as ♦ and chains in the ordered chain state as ●. The three plots are not given to scale. The **so–lo** phase line corresponds to the sub-main phase transition and the **lo–ld** phase line corresponds to the main phase transition.

characterized by high degree of chain order and lateral disorder. At a lower temperature, $T_{s-m} = 1.165V_2/k_B$, a low latent heat transition, which we will identify as the sub-main transition, takes the system from the **lo** phase into an **so** phase characterized by a high degree of order in both the translational and the chain variables. The two transitions can be established to be of first order (Nielsen, M., Miao, L., Ipsen, J.H., Mouritsen, O.G., and Zuckermann, M.J., unpublished).

The latent heat or transition enthalpy, ΔH_{s-m}, for the sub-main transition can be found by integrating the specific heat curve. An estimate of the corresponding transition entropy per chain, $\Delta S_{s-m} = \Delta H_{s-m}/T_{s-m}$, across the transition gives a value of approximately 0.45 k_B. ΔS_m

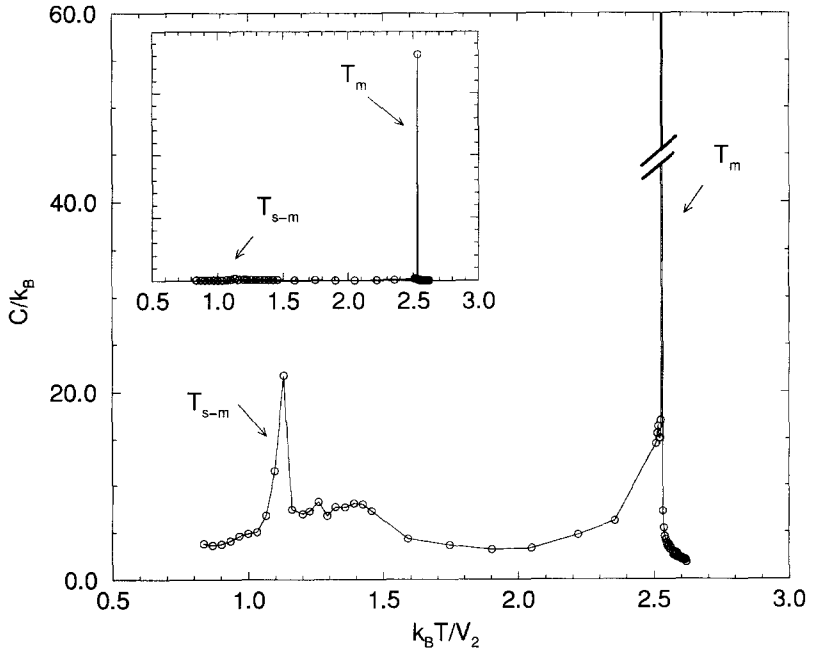

Fig. 3. Theoretical specific heat, $C(T)$ for a lipid material characterized by a specific value of the model parameter $V_1/V_2 = 0.5$, cf. the phase diagram in Fig. 2. Since the specific heat peak is much more intense at the main transition than at the sub-main transition, $C(T)$ is shown in full scale in the inset and on a broken scale in the main figure in order to reveal as many details as possible.

associated with the main transition is found to be of the order 14–15 k_B, and the latent heat of the sub-main transition is thus only a few percent of the latent heat in the main transition.

As we vary the value of V_1/V_2 and move towards the triple point, the position of the sub-main transition is shifted to higher temperatures. Our simulations show a systematic dependence of ΔS_{s-m} on the model parameter V_1/V_2. Indeed, as we move along the **lo–so** phase boundary towards the triple point we find a steady increase in the sub-main transition entropy ΔS_{s-m} whereas ΔS_m stays approximately constant. Along the **lo–so** line, ΔS_{s-m} varies typically in the range 0.35 k_B to 0.8 k_B.

5. Discussion and comparison with experiment

In the recent high-sensitivity calorimetric study by Jørgensen [5] a sub-main transition was found to be present in fully hydrated multi-lamellar bilayers of long-chain lipids in the homologous series DCnPC, with $17 \leq n \leq 20$. The transition entropy per lipid molecule, ΔS_{s-m}, was found to be very small, being in the range $0.22\ k_B \leq \Delta S_{s-m} \leq 0.56\ k_B$. Both the transition temperature, T_{s-m}, and ΔS_{s-m} were found to display a systematic chain-length dependence [5]. In fact for increasing chain length, T_{s-m} moves towards T_m. The experimental data obtained by differential scanning calorimetry for the specific heat in the case of multilamellar vesicles of DSPC is shown in Fig. 4.

As described in Section 4 the present minimal model is, for appropriate values of the parameter, V_1/V_2, capable of producing two phase transitions as observed experimentally. Moreover, the model predicts, in qualitative agreement with the experimental observations, that the two transitions move towards each other and merge as the lipid acyl-chain length is increased. Due to the simplicity of the model it is not possible in a quantitative manner to relate the value of V_1/V_2 to a particular lipid. However, our model offers a simple but plausible explanation of the experimental data. Despite its simplicity, the proposed model is able to provide the values of the transition entropies of both the sub-main and the main phase transitions which are close to the experimental data. In particular, the prediction from the model, that the enthalpy change across the sub-main transition is only a few percent of that of the main transition, can be regarded as the single most important piece of evidence that the minimal model has captured the essential physics of the experimentally observed transitional behavior of the sub-main transition. It is obvious that by a suitable choice of V_1/V_2 it is possible to arrange that the calculated difference between the two transition temperatures is close to the experimental value for a particular lipid material, e.g. DSPC. We have not made any attempt to fit the model parameters to any particular lipid material, but rather have given the generic result for the sake of clarity. In the case of using the model parameters pertinent to e.g. DSPC the close proximity of the transitions would make it computationally very demanding to separate the two contributions to the specific heat.

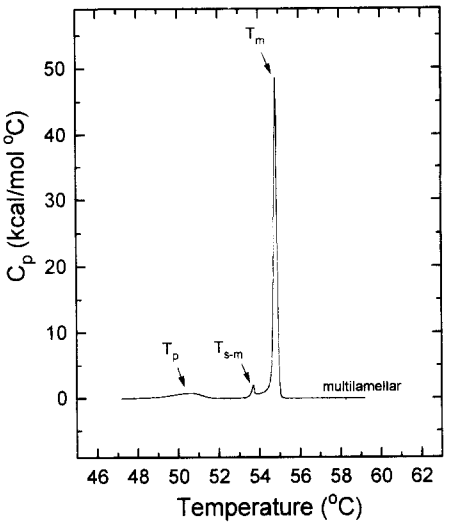

Fig. 4. Experimental specific heat, $C(T)$, for fully hydrated DSPC multi-lamellar vesicles obtained by differential scanning calorimetry at a scan rate of 3.6°C/h using a MicroCal MC-2 (Northampton, MA). The DSPC lipid was purchased from Avanti Polar Lipids (Birmingham, AL). The data displays a pretransition at $T_p = 50.6$°C, a submain transition at $T_{s-m} = 53.8$°C, and a main transition at $T_m = 54.8$°C. The transition entropies for the sub-main transition and the main transition are 205 cal/mol and 10.9 kcal/mol respectively.

For decreasing chain length, the experimental data [5] show that the sub-main transition vanishes or becomes undetectable when the chain length becomes less than $n = 17$. It is well known, that as the acyl-chain length is decreased, the lipid-bilayer density fluctuations near the main phase transition become stronger corresponding to the approach to a critical point [18,20]. Within the present microscopic model the approach to a critical point indeed implies that the specific-heat peak of the main transition is broadened substantially (Nielsen, M., Miao, L., Ipsen, J. H., Mouritsen, O. G., and Zuckermann, M. J., unpublished). This may provide an explanation of the absence of any sub-main specific heat anomaly. The sub-main transition is simply washed out by the near-critical fluctuations of the main transition.

The success of the present minimal model to rationalize experimental data for the sub-main phase transition in terms of a decoupling mechanism related to lattice melting and acyl-chain melting transitions strongly suggest that it is desirable to perform wideangle X-ray or neutron-scattering experiments with a sufficient temperature resolution near the main phase transition temperature for multi-lamellar DSPC vesicles to establish whether the crystalline order of the bilayers is lost or not before the chain melting at the main transition sets in.

Acknowledgements

This work was supported by the Danish Natural Science Research Council, the Danish Technical Research Council, by the FCAR du Quebec under a centre and team grant, and by NSERC of Canada. M.N. acknowledges support from the Danish Research Academy. O.G.M. is an Associate Fellow of the Canadian Institute for Advanced Research.

References

[1] Chen, S.C., Sturtevant, J.M. and Gaffney, B.J. (1980) Proc. Natl. Acad. Sci. USA 77, 5060–5063.
[2] Füldner, H.H. (1981) Biochemistry 20, 5707–5710.
[3] Slater, J.L. and Huang, C. (1987) Biophys. J. 52, 667–670.
[4] Gershfeld, N.L. (1989) Biochim. Biophys. Acta 988, 335–350.
[5] Jørgensen, K. (1995) Biochim. Biophys. Acta 1240, 111–114.
[6] Mouritsen, O. G. (1991) Chem. Phys. Lipids 57, 179–194.
[7] Ipsen, J.H., Karlström, G., Mouritsen, O.G., Wennerström, H. and Zuckermann, M.J. (1987) Biochim. Biophys. Acta 905, 162–172.
[8] Ipsen, J.H., Mouritsen, O.G. and Zuckermann, M.J. (1989) Biophys. J. 56, 661–667.
[9] Zuckermann, M.J. and Mouritsen, O.G. (1987) Eur. Biophys. J. 15, 77–86.
[10] Mouritsen, O.G. and Zuckermann, M.J. (1987) Chem. Phys. Lett. 135, 294–298.
[11] Ipsen, J.H., Mouritsen, O.G. and Zuckermann, M.J. (1989) J. Chem. Phys. 91, 1855–1865.
[12] Bloom, M., Evans, E. and Mouritsen, O.G. (1991) Quart. Rev. Biophys. 24, 293–297.
[13] Fraser, D.P., Zuckermann, M.J. and Mouritsen, O.G. (1991) Phys. Rev. A 43, 6642–6656.
[14] Doniach, S. (1978) J. Chem. Phys. 68, 4912–4916.
[15] Mouritsen, O.G. (1990) in Molecular Description of Biological Membrane Components by Computer Aided Conformational Analysis, Vol. 1 (Brasseur, R., ed.), pp. 3–83, CRC Press, Boca Raton, FL.
[16] Dammann, B., Fogedby, H.C., Ipsen, J.H., Jeppesen, C., J rgensen, K., Mouritsen, O.G., Risbo, J., Sabra, M.C., Sperotto, M. and Zuckermann, M.J. (1995) in Nonmedical Applications of Liposomes (Barenholz, Y. and Lasic, D., eds.), pp. 85–128, CRC Press, Boca Raton, FL.
[17] Mouritsen, O.G. (1984) Computer Studies of Phase Transitions and Critical Phenomena, Springer Verlag.
[18] Ipsen, J.H., Jørgensen, K. and Mouritsen, O.G. (1990) Biophys. J. 58, 1099–1107.
[19] Pink, D.A., Green, T.J. and Chapman, D. (1980) Biochemistry 19, 349–356.
[20] Morrow, M.R., Whitehead, J.P. and Lu, D. (1992) Biophys. J. 63, 18–26.