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The effects of acyl chain ordering and crystallization on the main phase transition of wet lipid bilayers

A theoretical study

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Abstract. The main gel-fluid phase transition of wet lipid bilayers is examined in terms of a microscopic interaction model which incorporates both *trans*-gauche isomerism of the lipid acyl chains and crystal orientation variables for the lipid molecules. The model gives two scenarios for the phase behavior of wet lipid bilayers in terms of temperature: *(i)* chain melting occurs at a higher temperature than crystallization, or *(ii)* chain melting and crystallization occur at the same temperature. Experimental data for lipid bilayers is consistent with the second scenario. In this case, computer simulation is used to investigate the non-equilibrium behaviour of the model. The numerical data is intepreted in terms of interfacial melting on heating and grain formation on cooling through the main phase transition. Interfacial melting is a non-equilibrium process in which the grains of a polycrystalline bilayer melt inwards from the boundaries. The prediction of interfacial melting in wet lipid bilayers is examined in relation to data from both equilibrium and non-equilibrium measurements, to corresponding phase behavior in monolayers, and to previous theoretical work.

Key words: Lipid bilayer, acyl chain ordering, crystallization, grain-boundary formation, interfacial melting

Abbreviations: DHPE – dihexadecyl phosphatidylethanolamine; DMPA – dimyristoyl phosphatidic acid; DMPC – dimyristoyl phosphatidylcholine; DPPC – dipalmitoyl phosphatidylcholine; DSC – differential scanning calorimetry; MCS/S – Monte Carlo steps per site

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Introduction

The dominant mechanism for the main gel-fluid phase transition of wet lipid bilayers is considered to be the "melting" of the acyl chains of the lipid molecules (for a review, see Quinn and Chapmann 1980), i.e. acyl chains undergo considerable *trans*-gauche isomerism during the main phase transition so that they pass from a rather rigid conformation dominated by *trans* bonds to a more flexible conformation with several gauche bonds (for a review, see Caillé et al. 1980). This has led to the construction of theoretical models of the main transition, which involve careful descriptions of the important rotational conformers of the acyl chains, their selection as to suitabilty and their interactions, but no other effects are treated in any detail. One important effect is the melting of the crystalline gel phase bilayer into a quasi-two-dimensional liquid, which occurs at the same time as the "chain melting" phenomenon. Upon reduction of the temperature below the melting point, the bilayer crystallizes into a quasi-two-dimensional lattice of acyl chains.

The reason for the previous neglect of the crystallization phenomenon is that it has a minor effect on the thermodynamic properties of the lipid bilayer when it is in equilibrium. Doniach (1978) estimates that its contribution to the enthalpy of transition is $\sim$ 1 kcal/mole and independent of lipid chain length, whereas the total enthalpy of transition is chain length dependent and 8.7 kcal/mole for hydrated dipalmitoyl phosphatidylcholine (DPPC) multi-bilayer systems. In addition, Melchior et al. (1982) have deduced from differential scanning calorimetry (DSC) data that the thermodynamics of several bilayer systems composed of pure lipids does not change with the rate of heating or cooling of the sample, and that no supplementary effects (e.g. a glass transition) occur as a result of increased heating or cooling rates. However, the most impor-

tant probes for the structure of lipid multi-bilayers are static x-ray diffraction (Costello and Gulik-Krzywicki 1976) and dynamic synchrotron x-ray studies which examine time-dependent phenomena at the main transition (Caffrey and Bilderback 1984; Caffrey 1985). Both these studies give information concerning the microstructure of wet lipid systems. The question arises: how does the crystallization/melting phenomenon affect the phase behavior of the bilayer?

To answer this question, we examine the case of a system with both a first-order lattice-melting transition and a first-order chain-melting transition, using a microscopic interaction model. When the lattice-melting temperature, T_m, is less than the chain-melting temperature, T_c, a polycrystalline sample of the system will first melt into a disordered phase in which the molecules will have the same chain conformations as in the crystalline phase, but in-plane long-range order is absent. On further heating, the system will pass through the chain-melting transition at T_c, which is characterized by strong conformational changes in the molecules. When the system is rapidly quenched through the lattice-melting transition, a polycrystalline solid forms.

In this article, we present theoretical results which show that, if the interactions which control the lattice-melting transition are sufficiently large, the chain-melting transition and the lattice-melting transition occur at the same temperature, T_{cm}. In equilibrium the combined transition is found to be an abrupt first-order phase transition. The non-equilibrium behavior of the system is investigated in detail for this case. We show that, when the system is quenched through T_{cm}, crystalline grains grow at the expense of the fluid phase in which the molecules are in a different molecular configuration. If the polycrystal is then heated sufficiently rapidly, we find that the molecules at the grain boundaries will change their conformations before the molecules in the interior of the grains and the grains will melt inwards from the boundaries into the fluid phase. This phenomenon is known as interfacial melting (Mouritsen and Zuckermann 1987 a) and is a non-equilibrium process, whose details depend on the rate of heating, or, in the reverse process of grain formation, on the rate of cooling. It is our contention that lipid bilayers are likely candidates for pronounced interfacial melting and grain formation in the neighbourhood of the main phase transition. However, a possibility which cannot be discounted is that the chain-melting transition and the lattice-melting transition of lipid bilayers occur at temperatures which are almost equal, but cannot be distinguished by equilibrium measurements. In this case the grains will again decrease inwards from their boundaries but with a disordered gel phase forming at the boundaries during the lattice melting transition. The chain melting will then begin at the higher temperature and, if the system is heated fast enough, the chain melting may begin before all the crystallites have vanished.

The outline of the paper is as follows: Firstly, we present a mathematical model for lipid bilayer phase transitions in terms of two Hamiltonians, one describing the conformational changes of the acyl chains and the other the formation and melting of grains. Secondly, a description is given of the results of calculations for this model as obtained from mean-field theory and from computer simulations. Lastly, these results are discussed in relation to experimental evidence and previous theoretical work. Of particular interest is the use of the model to distinguish between monolayer and bilayer melting phenomena.

Theoretical model

In order to study crystallization/melting phenomena in lipid bilayers, we have constructed a model on a triangular lattice. This model takes into account

(i) the internal conformational states of the acyl chains and their mutual interactions,
(ii) the large number of in-plane orientations of two-dimensional lipid crystallites.

The total Hamiltonian for the model is written:

$$H = H_L + H_P . \tag{1}$$

H_L is the Hamiltonian for Pink's ten-state model (Pink et al. 1980), which is specifically constructed to describe the main transition of one-component bilayers in terms of the conformational degrees of freedom of the acyl chains. Within the scope of this model, the bilayer is considered as two monolayers which are independent of each other. Each monolayer is represented by a two-dimensional triangular lattice with $N = L \times L$ lattice sites. Every site of the lattice is occupied by a single saturated hydrocarbon chain which is in one of ten distinct conformational states. Each state is characterized by an internal energy E_n, a cross-sectional area A_n, and a degeneracy D_n, where $1 \leqq n \leqq 10$. All ten states are derivable from the all-*trans* state in terms of *trans*-gauche isomerism. The two key conformational states are the non-degenerate gel-like ground state ($n = 1$) representing the all-*trans* conformation and a highly degenerate excited state ($n = 10$) characterizing the "melted" or fluid phase. The model is completed by including eight intermediate gel-like states which

contain kink and jog excitations. These intermediate states were selected by Pink (Pink et al. 1980) subject to the requirement of low conformational energy and optimal packing. The values of E_n are determined from the energy required for a gauche rotation (0.45×10^{-13} erg) relative to the all-*trans* conformation. The values of D_n are obtained from combinational considerations and A_n is calculated using the geometrical constraint that the volume of an acyl chain is invariant (Marčelja 1974). The chains interact via anisotropic forces which represent both van der Waals and steric interactions. The lattice approximation automatically accounts for the excluded volume effects and an internal lateral pressure of 30 dyne/cm is used to model the interfacial forces required for bilayer stability. The model is used here with parameters appropriate for DPPC and the reader is referred to Pink et al. (1980) for the mathematical form of H_L. It should be pointed out that although the Pink model is arrayed on a lattice, there is an implicit distance dependence in the chain-chain interactions via the cross-sectional areas.

The second term, H_P, of the Hamiltonian is a modification of the high-q-state Potts model, where q is the number of Potts states. The standard-q-state Potts model is a lattice model which has been successfully used to describe grain growth in polycrystalline aggregates (Anderson et al. 1984). The standard Potts model accounts for the grain-boundary energy of a metastable distribution of crystalline domains, each of which is characterized by a Potts state. In the modified Potts model, only the first nine conformational states carry a Potts variable which then describes the orientation of the crystalline domain with which the chains are associated. When the conformational state of a chain changes from a gel-like conformer to the excited (10th) state, it loses its Potts variable, which gives rise to zero grain-boundary energy. This is reasonable since the excited state is representative of the fluid phase of the bilayer, which cannot be in a granular configuration. The grain-boundary energy is modelled by allowing neighbouring acyl chains to interact with an energy $J_P > 0$ if they are in different Potts states. Otherwise the interaction is zero. H_P accounts in a very approximate way for the phenomenon of crystallization which of course in the real system takes place in terms of translational degrees of freedom. By describing crystallization by a Potts model on a lattice, we are unable to account for more subtle two-dimensional effects (Mouritsen and Zuckermann 1987 a, b). H_P can be written as follows:

$$H_P = J_P \sum_{\langle ij \rangle} \sum_{n,n'=1}^{9} \sum_{p,p'=1}^{q} (1 - \delta_{pp'})\, L_{pn}^{(i)}\, L_{p'n'}^{(j)}, \qquad (2)$$

where i and j are lattice-site indices ($i, j = 1, \ldots N$) and $L_{pn}^{(i)}$ are state projection operators for the ith lattice site, i.e.

$$L_{pn}^{(i)} = 1 \quad \text{ith chain in nth conformational state and pth Potts state}$$
$$= 0 \quad \text{otherwise} \qquad (3)$$

$\langle ij \rangle$ denotes that i and j are nearest neighbours on the lattice.

A "stripped-down" version of the model in Eq. (1) has been used to investigate interfacial melting per se (Mouritsen and Zuckermann 1987 a) and the model has also been used in conjunction with a term describing an external lateral pressure to account for the formation of crystallites in lipid monolayers at an air/water interface (Mouritsen and Zuckermann 1987 b).

The choice of an appropriate value of q is made on the basis of previous computer simulations for the standard Potts model (Sahni et al. 1983). It was found that the limit $q \to \infty$ corresponding to infinitely many crystal orientations is adequately described by choosing $q \geqq 30$. The value $q = 30$ is therefore used in our calculations.

Calculations and results

Mean-field approximation

The phase behavior described by the conformational part of the Hamiltonian, H_L, is well understood and has been the subject of several papers (see e.g. Mouritsen and Zuckermann 1985 and references therein). The inclusion of the modified Potts Hamiltonian changes the equilibrium phase behavior and a good guide to such changes can be obtained from using the Hamiltonian of Eq. (1) in conjunction with the mean-field approximation. The calculational details are similar to those given in previous work (Pink et al. 1980) and only the results are presented.

Figure 1 gives the phase diagram for the Hamiltonian of Eq. (1) in terms of the temperature, T, and the Potts interaction constant, J_P. The value of J_0, the coupling constant of the interaction between chain conformational states, is chosen as 1.32×10^{-13} ergs. For small values of J_P, three distinct phases occur:

A crystalline gel phase at low temperatures. Here the lipid chains are mostly gel-like (i.e. predominantly in one of the nine gel-like conformational states) and the phase is characterized by a single Potts state. The crystalline gel phase is interpreted as a structurally ordered solid phase described by a two-dimensional lattice.

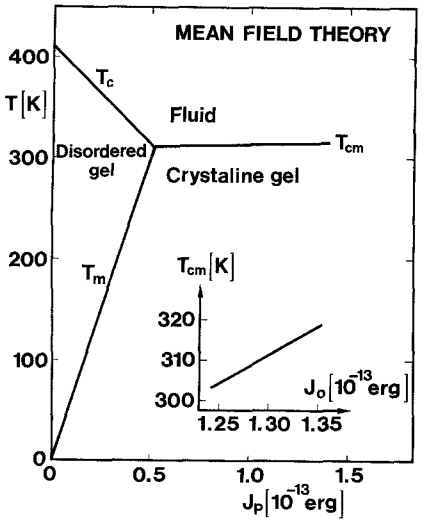

Fig. 1. Mean-field phase diagram for the lipid bilayer model of Eq. (1) in terms of temperature T and grain-boundary energy constant J_P (Potts interaction strength). The coupling constant between lipid chain conformational states is $J_0 = 1.32 \times 10^{-13}$ ergs. Three first-order phase boundaries divide the phase diagram into a fluid, a crystalline gel, and a disordered gel region. The equilibrium transition temperatures are denoted T_c for the acyl chain ordering transition, T_m for the two-dimensional crystallization, and T_{cm} for the combined chain ordering and crystallization process. The *insert* shows the variation of T_{cm} with the grain-boundary energy constant J_P. For $J_0 = 1.32 \times 10^{-13}$ ergs the transition temperature is $T_{cm} = 314$ K corresponding to the main transition of DPPC bilayers

A disordered gel phase at intermediate temperatures, in which the lipid chains are again gel-like, but there is a random distribution of Potts states over the lattice. This phase is interpreted as a structurally disordered solid phase with slow in-plane lateral diffusion and no long-range order (i.e. a glass phase).

A fluid phase at high temperatures. Here the chains are mostly in the excited conformational state and the few remaining gel-like chains are Potts disordered. The fluid phase is interpreted as a structurally disordered fluid phase, exhibiting fast in-plane lateral diffusion.

All phase boundaries represent first-order phase transitions. As J_P increases, the crystalline gel phase becomes more favoured and the transition temperature, T_{OD}, for the disordered gel-crystalline gel transition increases. At the same time, the transition temperature, T_{GF}, for the disordered gel-fluid transition decreases since J_P acts like a repulsive interaction between chains in gel-like conformational states and in different Potts states. We identify T_{OD} as the crystallization temperature, T_m, and T_{GF} as the transition temperature, T_c, at which chain melting occurs.

When J_P reaches a critical value J_P^* ($\sim 0.5 \times 10^{-13}$ ergs in mean-field theory), the intermediate disordered gel phase disappears at a triple point. For values of $J_P > J_P^*$ only one phase transition occurs between the crystalline gel and the fluid phases. We can identify this transition as the main transition in the case when crystallization and "chain melting" occur simultaneously. The phase boundary of this transition is almost invariant with respect to J_P for $J_P > J_P^*$ and an insert in Fig. 1 shows the sensitivity of the transition to J_0 for varying J_P. It can be seen from this insert that, when $J_P = 10^{-13}$ ergs and $J_0 = 1.32 \times 10^{-13}$ ergs, the main transition is fixed at its value for DPPC (314 K).

Computer simulations: Description

Since the mean-field approximation applies to equilibrium phenomena only, the non-equilibrium behavior resulting from the model is investigated by computer simulations using a Monte Carlo importance-sampling procedure (Mouritsen 1984). Equilibrium Monte Carlo calculations are more accurate than mean-field calculations since they take proper account of the thermal fluctuations suppressed in the mean-field approximation. In addition, they can be used to investigate the non-equilibrium behavior of the Hamiltonian, since the physical variables of the system are calculated as functions of both temperature and time. Time is measured in terms of Monte Carlo steps per site (MCS/S), where one Monte Carlo step is one attempted excitation per site (Mouritsen 1984). Here we use the Monte Carlo procedure to generate the numerically exact solution to the statistical mechanical problem of an interacting system of lipid chains, each characterized by ten conformational states and 30 Potts states. A computer simulation of this type has several advantages over conventional theory. Firstly no mathematical approximations to the model are required. Secondly, the simulation gives access to the microscopic configurations of the bilayer system, thereby giving a visual display of the melting processes. Thirdly, Monte Carlo computer simulations may be thought of as numerical experiments on a well-defined system, undertaken under completely controlled conditions and the numerical results can be analysed in the same manner as experimental data.

The Monte Carlo calculations are performed on finite lattices subject to periodic boundary conditions. Effects due to finite size are estimated by comparing results for two different system sizes, $N = 900$ and $N = 10{,}000$. The calculations are carried

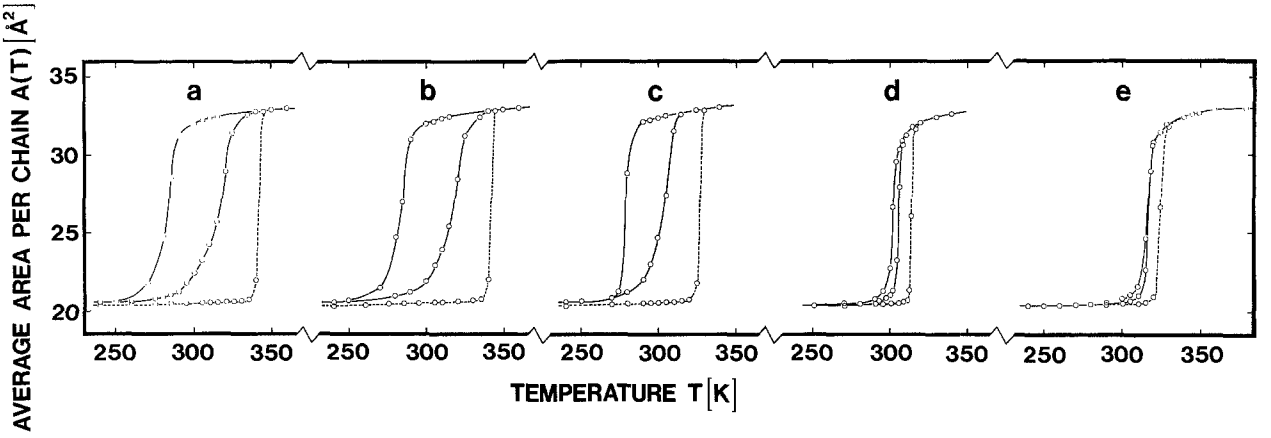

Fig. 2a–e. Computer-simulation results for the average cross-sectional area per lipid chain as a function of temperature. The calculations are carried out on systems with N chains, and the equilibration time at each temperature is M MCS/S. The coupling constant between lipid chain conformational states is in all cases $J_0 = 1.32 \times 10^{-13}$ ergs. The grain-boundary energy constant is J_P. **a** $N = 900$, $M = 1{,}000$, $J_P = 10^{-13}$ ergs; **b** $N = 10{,}000$, $M = 1{,}000$, $J_P = 10^{-13}$ ergs; **c** $N = 900$, $M = 2{,}000$, $J_P = 10^{-13}$ ergs; **d** $N = 10{,}000$, $M = 4{,}000$, $J_P = 0.55 \times 10^{-13}$ ergs; **e** $N = 10{,}000$, $M = 4{,}000$, $J_P = 0.525 \times 10^{-13}$ ergs

out for increasing as well as decreasing temperature in order to examine hysteresis effects.

The systematics of the simulations are as follows: the initial configuration of the lattice is chosen to be an equilibrium configuration corresponding to the crystalline gel phase, i.e. every chain is put into the all-*trans* conformational state ($n = 1$) with the same Potts variable, more precisely the ground state of the system. The value of J_0 is fixed and the value of J_P is chosen to be greater than or close to J_P^*. An initial temperature below the melting temperature is chosen and the system is equilibrated to an equilibrium state of the crystalline gel phase. The temperature is then increased in small increments, with the same number of MCS/S performed at each temperature, until the system suffers an abrupt transition to the fluid phase. This gives the upper limit of the hysteresis loop of the crystalline gel-fluid transition. The temperature is then decreased so that the system passes from the fluid phase to the crystalline gel phase and then increased a second time so as to take the system back to the fluid phase. These procedures are implemented in order to investigate non-equilibrium effects near the main phase transition.

Computer simulations: Results

The computer simulations monitor the phase of the bilayer by calculating the average area per chain as a function of temperature and time. A low area per chain, A ($\lesssim 22\,\text{Å}^2$), implies that the system is in the gel phase whereas a high area per chain ($\gtrsim 30\,\text{Å}^2$) signifies a fluid phase (Pink et al. 1980). Positions of phase boundaries are estimated using standard techniques (Mouritsen 1984). The phase diagram determined by the simulations is qualitatively similar to the mean-field phase diagram of Fig. 1, except that the phase boundaries are at slightly different positions. In particular, the more accurate computer simulation result of $J_P^* \cong 0.535 \times 10^{-13}$ ergs is below the mean-field estimate.

Figure 2 shows the behavior of A versus the temperature for $J_0 = 1.32 \times 10^{-13}$ ergs and several values of J_P. We chose a high value of J_P ($J_P = 10^{-13}$ ergs) to begin with, so as to exaggerate the hysteresis effects and obtain a clear picture of the non-equilibrium phenomena occuring in lipid bilayer systems. Figure 2a gives the A versus T curves for $J_P = 10^{-13}$ ergs, $N = 900$ sites, temperature steps of 5 K and 1,000 MCS/S at each temperature. The abrupt transition from the crystalline gel to the fluid phase occurs at $T = 335$ K for the first series of increasing temperatures. When the temperature is decreased from 340 K, the system remains in the fluid phase until $T = 300$ K, after which the phase changes continuously to the crystalline gel phase over a range of 50 K. However, the area per chain in this gel phase is slightly larger than that found during the initial run. When the temperature is increased for the second time, the area per chain increases continuously with temperature, the path chosen by the system being completely different from either of the two previous runs.

The simulation is repeated with the same parameters but for a lattice dimension of $N = 10{,}000$ sites. The results are shown in Fig. 2b and the curves of A versus T are found to be almost identical to the results of Fig. 2a for the smaller lattice

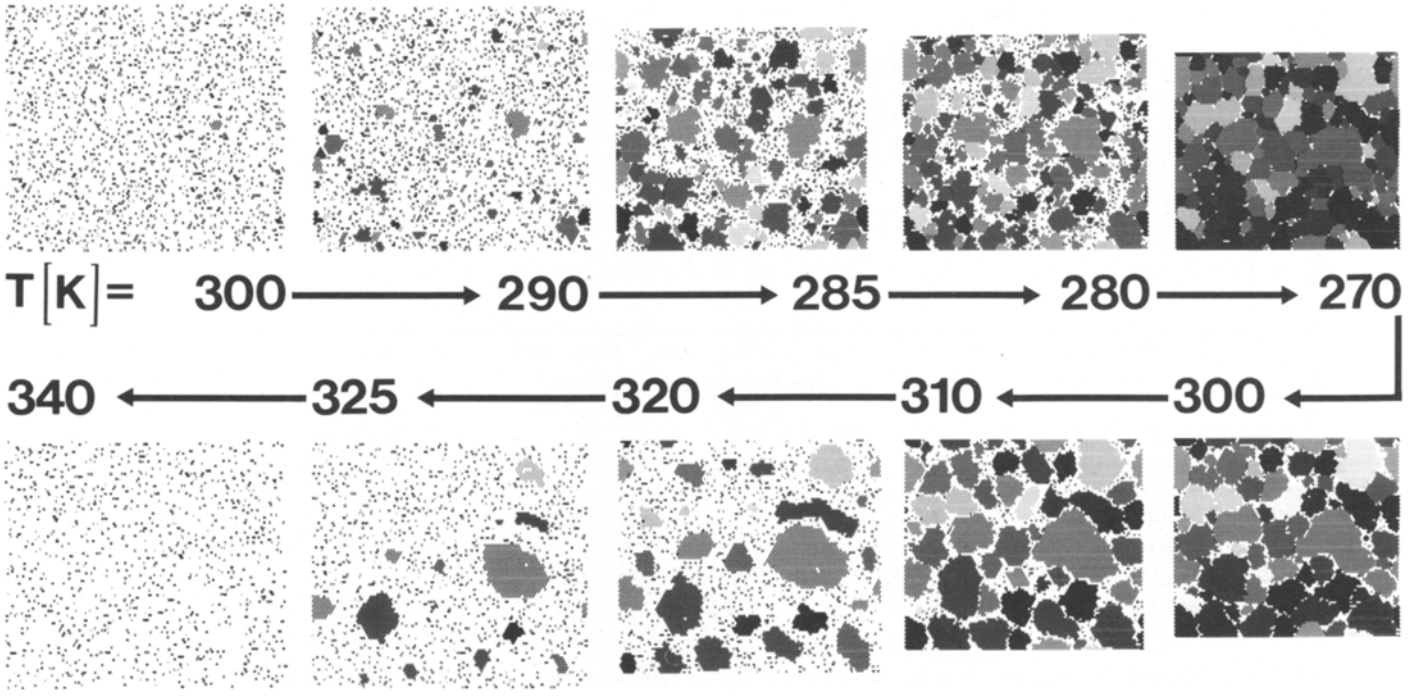

Fig. 3. Snapshots of microconfigurations illustrating the non-equilibrium processes of grain-boundary formation and subsequently interfacial melting (Fig. 2b) for a system with 10,000 lipid chains. White areas indicate fluid domains and symbols indicate lipid molecules in gel conformations, with each symbol labelling a crystal-orientational state (Potts state) of the lipid molecule. The lattice parameter has been scaled so as to display the thermally induced area contraction and expansion of the lipid bilayer

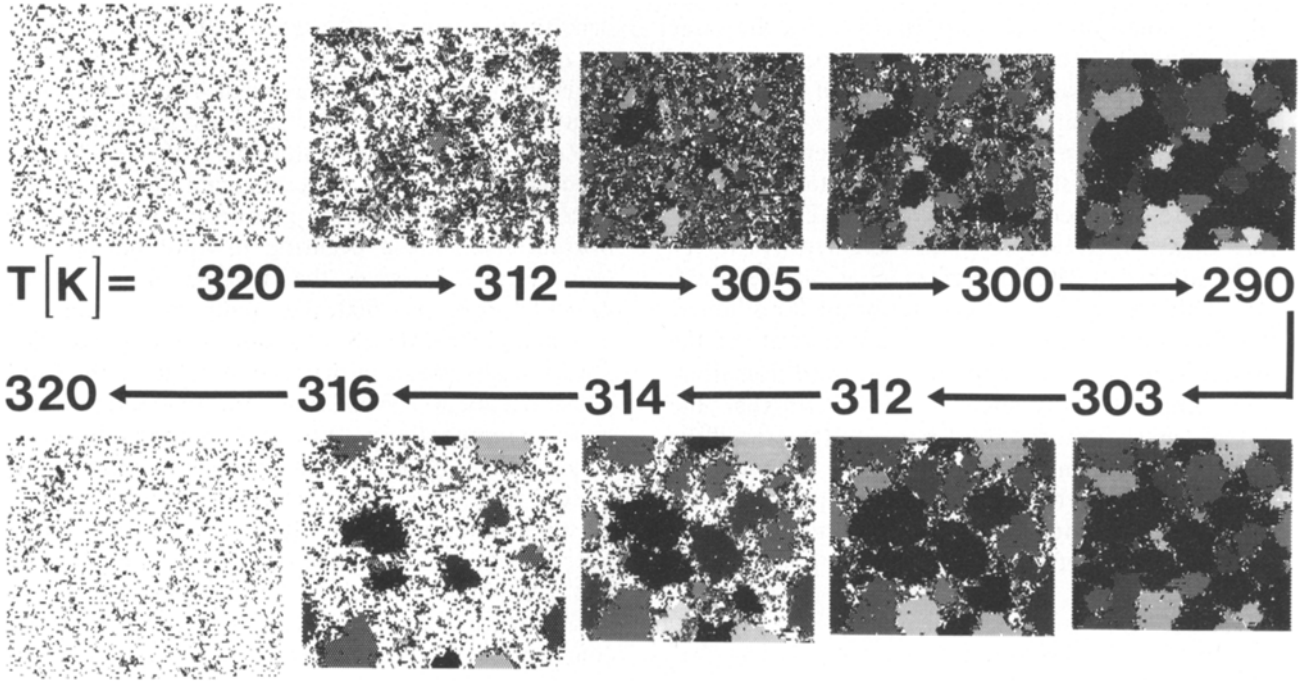

Fig. 4. Same as Fig. 3 for the situation displayed in Fig. 2d

sample, indicating the absence of significant size effects. Snapshots of micro-configurations corresponding to selected points on the curves in Fig. 2b are shown in Fig. 3. From the snapshots it can be seen that grains of the crystalline gel phase begin to grow at the expense of the fluid phase when the temperature is first decreased. On further decrease of temperature the grains take over the lattice, pushing out the interfacial fluid, so that the system is almost entirely granular at 270 K. When the tem-

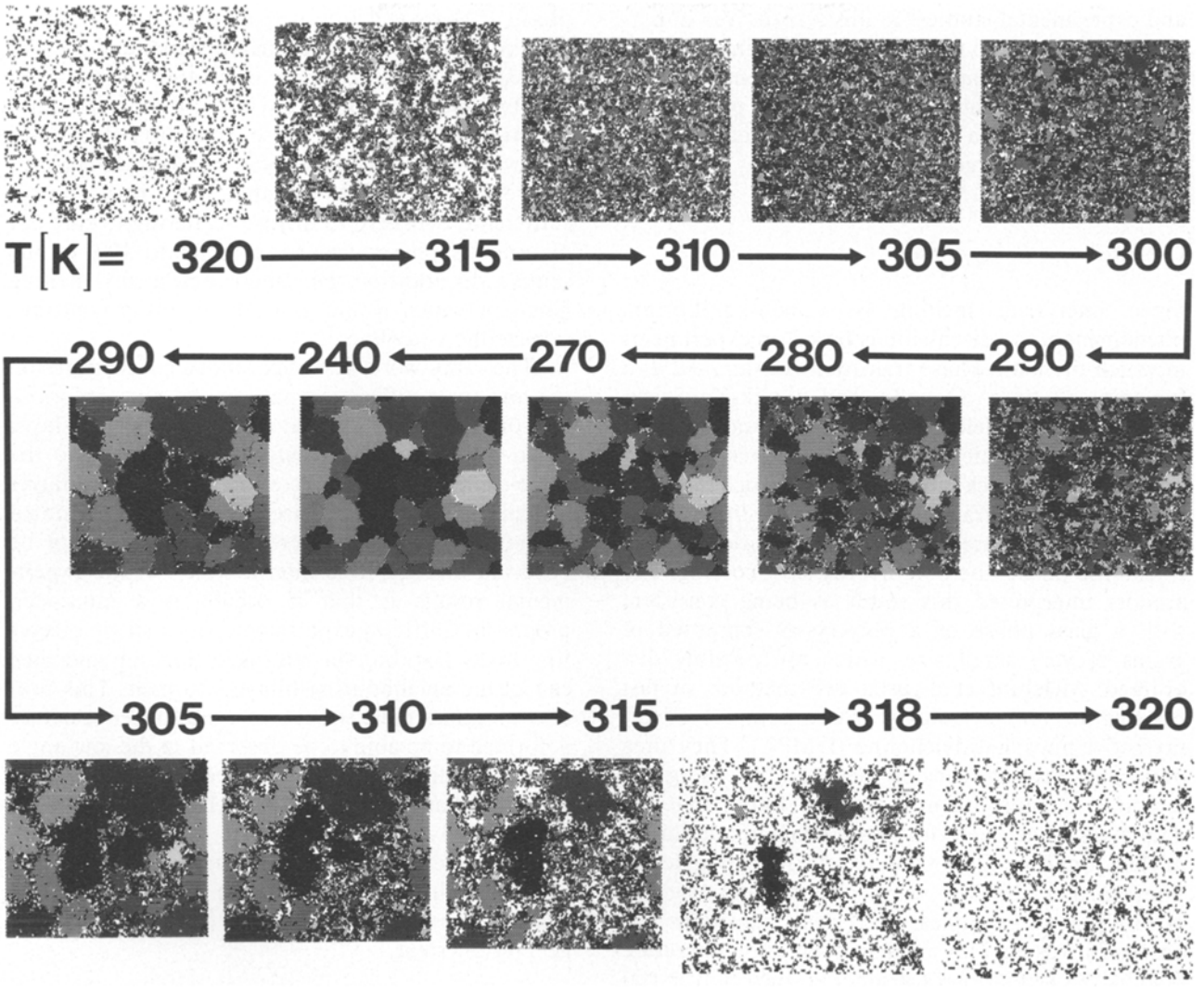

Fig. 5. Same as Fig. 3 for the situation displayed in Fig. 2e. In this case $J_P < J_P^*$ and an intermediate disordered gel phase occurs between the crystalline gel phase and the fluid phase. Interfacial melting in the sense of Figs. 3 and 4 is absent but the polycrystalline gel disorders at increasing temperatures by formation of a glassy interface at the domain boundaries

perature is now increased, interfacial melting occurs with the system melting uniformly from the grain boundaries inwards. The interfacial nature of the melting can be seen quite clearly from the snapshot at 310 K. The snapshots indicate that interfacial melting is considerably more uniform than grain formation in our model.

Figures 2a, b and 3 show that the hysteresis in the interfacial melting/grain formation processes takes place over a broad temperature range from our choice of J_P and the number of MCS/S. The width of the hysteresis decreases when the number of MCS/S is increased at each temperature as shown in Fig. 2c. Since we are observing a non-equilibrium phenomenon, the interfacial melting will no longer occur if enough MCS/S are used so that equilibrium is attained at every temperature (see Mouritsen et al. 1983; Mouritsen and Zuckermann 1985). Another way of reducing the hysteresis width is to decrease the value of J_P towards J_P^*. Figure 2d gives A versus T curves from Monte Carlo calculations for $J_P = 0.55 \times 10^{-13}$ ergs. The hysteresis effects are considerably narrowed, but interfacial melting is still present, as seen in the snapshots of Fig. 4. Finally, Fig. 2e shows the A versus T curves for $J_P = 0.525 \times 10^{-13}$ ($J_P \leqq J_P^*$) and the absence of interfacial melting is evident in the corresponding snapshots of Fig. 5.

Discussion

In this section, the results of the preceding section will be discussed in the context of related theoretical

and experimental studies. In this respect it is important to bear in mind that interfacial melting is a non-equilibrium effect and is therefore absent from the melting of lipid bilayers when it is possible to prepare them in a non-granular, annealed equilibrium state in the gel phase.

Comparison with experiment

Since interfacial melting is a non-equilibrium phenomenon, the discussion is based on experiments in which the main phase transition is examined as a function of time. Costello and Gulik-Krzywicki (1976) and later Melchior et al. (1982) examined the physical state of quick frozen multi-bilayers. Costello and Gulik-Krzywicki found that, for such systems, the wide-angle diffraction pattern of the lipid chains is broad but centred around the position of the crystalline phase line obtained by slow cooling. The authors interpreted this result as being consistent with a glass phase or a polycrystal composed of grains of very small size, which are possibly disordered. Melchior et al. used two methods of fast freezing to obtain quickly frozen samples of dimyristoyl phosphatidylcholine (DMPC). They then performed heating scans on these samples using differential scanning calorimetry and found that the data gives *no* evidence for phase behavior additional to that found on slow cooling and reheating scans. They further showed, using glycerol and calcium cardiolipin as examples, that glass transitions are observable using DSC. Their conclusion therefore favours the formation of a small grained polycrystal as opposed to a glass phase on quick freezing of pure lipid bilayers. However, it is important to note that a glass transition may well be too weak relative to the other transitions to be observed.

Caffrey and Bilderback (1984) have performed real-time synchrotron x-ray diffraction on DPPC multilayers with a view of characterizing the kinetics of the main phase transition. Caffrey (1985) performed the same experiments for dihexadecyl phosphatidylethanolamine (DHPE) in order to examine the kinetics of both the main phase transition and the fluid to inverted-hexagonal phase transition. DHPE was chosen because, unlike DPPC, it does not have an intermediate ripple phase ($P_{\beta'}$). This permits a detailed study of a phase transition between a non-tilted crystalline gel phase ($L_{\beta'}$) and the fluid phase (L_α). The samples were heated through the various phase transitions by hot air directed at the sample by a hot air gun. The low-angle diffraction pattern always gave sharp lines for both the gel and the fluid phase, before, during and after the transition. Upon heating the lipid in the gel phase, a sharp gel line was first observed. After a certain time a sharp fluid line appeared and grew at the expense of the gel line, indicating the presence of a two-phase region. We will not discuss the fluid to inverted-hexagonal phase transition since our theory is not constructed for non-bilayer phases. Caffrey (1985) points out that his x-rays are not sufficiently sensitive to diffuse scattering to rule out amorphous phases occurring in up to 10% of the sample. In addition, he cannot detect any melting behavior which would give low-intensity contributions to the x-ray lines.

The x-ray work described above concentrates on the low-angle diffraction pattern and gives information on the lamellar repeat distance in a multi-layer stack. This is therefore information relating to the three-dimensional character of the multi-bilayer system and the occurence of a two-dimensional phenomenon such as interfacial melting cannot be observed directly. One interpretation of the experimental results is that it occurs as a multi-step process in Caffrey's experiments, i.e. a single bilayer first melts possibly via interfacial melting and then causes the neighbouring bilayers to melt. This process continues until enough of a multilayer structure is formed to be able to be observed in the low-angle diffraction line. The quick frozen membranes, on the other hand, were made to change phase much more rapidly. This implies the presence of many bilayers in different stages of grain formation, but close enough to the gel phase to give a diffuse line near the position characteristic of a slowly cooled gel phase system.

Comparison with recent theoretical work

The ten-state Pink model in the absence of the Potts interaction has been studied in detail by means of computer simulations in the work of Mouritsen (1983), Mouritsen et al. (1983), and Mouritsen and Zuckermann (1985). Their results suggest that the first-order main phase transition, which behaves experimentally like a continuous transition, is explicable in terms of metastable states involving statistical clusters of lipid molecules which are closely related to the presence of strong thermal fluctuations due to the chain conformations. The inclusion of the Potts interaction, J_P, tends to sharpen the crystalline gel-fluid phase transition in the absence of grain boundaries, thereby reducing the extent of the large statistical clusters. The degree of the suppression of the thermal fluctuations depends on the values chosen for J_0 and J_P. When interfacial melting occurs, the conformational thermal fluctuations determine the conformational

states of the acyl chains within each domain or grain but are no longer entirely responsible for the non-equilibrium melting process itself.

The data from the computer simulations reported by Mouritsen et al. (1983) for the ten-state Pink model show considerable hysteresis at the main transition due to the presence of the statistical clusters, which represent long-lived metastable states. The inclusion of J_P requires an increase in the value of J_0 in order to ensure that the crystalline gel-fluid transition temperature remains equal to the main transition temperature of DPPC. The uniform crystalline gel-fluid phase transition therefore exhibits a wide hysteresis cycle with quite abrupt transitions. However, in the case of interfacial melting, the results of this paper show that the width of the hysteresis can be made to decrease by reducing J_P or the number of MCS/S at each temperature step so as to correspond to the experimental situation under examination.

Relationship to lipid monolayer properties

Kjaer et al. (1987) recently used synchrotron x-rays to investigate the structure of dimyristoyl phosphatidic acid (DMPA) monolayers spread on air-water interfaces. Their experiments indicate the presence of two phase transitions displaced by a lateral pressure of at least 15 dyne/cm. No Bragg reflections were observed close to the liquid expanded-liquid condensed phase transition or in the flat part of the isotherms. Bragg reflections were only observed in the very steep part of the isotherms. The Bragg peaks are quite broad and correspond to a correlation range of only $5-20$ molecular diameters. Mouritsen and Zuckermann (1987 b) showed that these experimental findings are in good agreement with the theory presented in the present work for $J_P \leqq J_P^*$. They pointed out that the liquid condensed-liquid expanded phase transition of monolayers corresponds to the disordered gel-fluid phase transition and the transition at higher lateral pressures along the steep part of the isotherm corresponds to the crystalline gel-disordered gel phase transition. A tentative conjecture is therefore that the model in Eq. (1) describes the phase behavior of lipid monolayers when $J_P < J_P^*$ and that of wet lipid bilayers when $J_P > J_P^*$. Support for this conjecture would come from high-resolution synchrotron x-ray diffraction data at wide angles, which gives the in-plane structure of multi-bilayer systems.

At present, we know of no direct way of estimating the value of J_P (for a particular lipid species) in terms of molecular properties. Also, it remains unclear whether J_P depends on the state of aggregation of the lipid molecules, be it a monolayer or bilayer. Further monolayer studies of the type reported by Kjaer et al. (1987) carried out systematically for a series of lipid species may serve to clarify these points.

Conclusions

The discussion above summarizes two classes of experiments from which evidence for interfacial melting and grain formation could be found. These are:

(a) Real-time synchrotron x-ray experiments of the type reported by Caffrey and Bilderback (1984) and Caffrey (1985). These experiments were performed using a temperature jump after which the system passed through the main phase transition at a rate of about 24 °C/min. However, for the observation of interfacial melting, the bilayer must initially possess a poly-crystalline structure and should be heated quite rapidly through the main phase transition.

(b) Experimental work on lipid bilayer systems which were rapidly quenched from the fluid phase to a crystalline gel phase. The quench rate is of the order of $1{,}000-3{,}000$ °C/min and the reason for so high a quench rate is to fix the initial state of the bilayer rather than to study the dynamics of the main phase transition.

We therefore propose that interfacial melting and grain formation are best detected when lipid bilayers are heated or quenched at rates intermediate to those used in *(a)* and *(b)* above and that real-time synchrotron x-ray diffraction at wide angles is a possible method of detection.

It is difficult at this stage to recommend specific lipid systems suitable for the observation of interfacial melting. A possibility is to use bilayers composed of lipids with phosphatidylethanolamine polar heads for which the P_β, phase is absent. However, it is conceivable that interfacial melting does occur when DPPC for example is rapidly heated from the $P_{\beta'}$ phase to the fluid phase. The grains will have different shapes from those shown in Fig. 3 and the interfaces between the $P_{\beta'}$ grains and the fluid phase will be controlled by the tendency of the lipid molecules to tilt with respect to the bilayer plane in the gel phase (Rüppel and Sackmann 1983). In this case interfacial melting can be detected from electron micrographs since structures characteristic of the $P_{\beta'}$ phase are typically three-dimensional (Rüppel and Sackmann 1983).

The model presented in this paper can also be used to examine the properties of impure and mixed

bilayer systems. For example, cholesterol not only removes the main phase transition, but also suppresses the ability of bilayers to form crystals. This implies within the model that an increase in the cholesterol concentration causes the effective value of J_P to fall below J_P^*, resulting in a second transition which is broadened by the presence of impurities. This problem is under active investigation.

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