Simulation of hydrogen bonding and hydration in pure lipid bilayers

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We propose a model for phase transitions involving hydrogen bonding in lipid bilayers. The model combines a five-state interacting hydrogen bonding model of the polar heads on a rectangular lattice with a ten-state model of the hydrocarbon chain states on a triangular lattice. Experimental data for the transition temperatures for the lipids dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylethanolamine (DMPE) are used to determine phenomenological parameters needed in Monte Carlo simulations of the lipid bilayers. The latent heats for the chain melting transitions of DMPC and DMPE are then computed in the simulations and compared with experiment.

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I. INTRODUCTION

Modeling of biological membranes is of considerable interest because of their interesting structural behavior and the information about biological function that may be obtained. The first step toward understanding biological membranes is to study the behavior of pure phospholipid bilayers which are considered to be structural models for biological membranes. The phospholipids themselves are surfactantlike molecules consisting of two hydrophobic fatty acid chains and a hydrophilic polar head. Two types of polar head, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), together account for the polar heads of the majority of phospholipids in most animal cell membranes [1]. The nature of the polar head is important for the hydration properties of the bilayer. Lamellar phases composed of PE hydrate less strongly than the corresponding lamellar phases of PC [2], and show a much greater tendency to form dehydrated and/or nonlamellar phases than do PC lipids of comparable acyl chain composition. There are three differences between PC and PE head groups. First, the volume of the PC head group is greater than that of PE. Second, the nonhydrogen atoms of the choline moiety form a branched structure in PC but a linear structure in PE. Finally, there is the possibility for hydrogen bonding between head groups of PE, but not of PC. It is likely that hydrogen bonding between the PE polar heads is responsible for the higher phase transition temperature of PE phospholipids compared to corresponding PC phospholipids [3, 4]. One of the goals of the present study is to quantify this effect.

The hydrogen bonding of dilauroyl phosphatidylethanolamine (DLPE) has been investigated by using x-ray crystallography by Hitchcock *et al.* [5] who found that in anhydrous crystals each PE polar head was connected by N—H...O hydrogen bonds to four neighboring polar heads. The hydrogen bonds are between the oxygens (acceptors) of the phosphate group and the hydrogens (donors) of the amino group. Since the trimethylammonio group of PC lipids cannot form hydrogen bonds, the PC head group can serve as an acceptor but not as a donor of hydrogen bonds.

Fully hydrated one-component PC bilayers undergo a phase transition, known as the main phase transition, in which the bilayer passes from a gel (solid) phase to a liquid-crystalline (fluid) phase. Both these phases are stable hydrated phases. For pure PE lipid bilayers, the situation is considerably different. Several studies [6–11] have shown that aqueous dispersions of dimyristoyl phosphatidylethanolamine (DMPE) can form at least three distinct types of lamellar phase: a stable, virtually dehydrated "crystalline" (AS) phase in which the acyl chains are rigid and tightly packed and the polar heads are presumably hydrogen bonded to one another; a hydrated solid (HS) metastable phase, in which the chains are somewhat less tightly ordered; and a hydrated fluid (HF) stable phase, in which the chains are flexible. The crystalline nature of the AS phase has been confirmed by the x-ray crystallographic studies of Seddon and coworkers [6, 7] who show that this phase has basically the same structure as the anhydrous crystal. On heating, the AS phase makes a first order phase transition to the HF phase at 330.5 K. This temperature is much higher than the chain melting transition temperature of DMPC at 296 K. Several effects occur at the AS to HF transition of DMPE. First, the solid melts and the acyl chains become flexible (chain melting). A second likely effect in the HS and HF phases is that the interlipid hydrogen bond network existing in the AS phase could be disrupted by competition with lipid-water interactions. The precise degree to which interlipid hydrogen bonding in-

teractions are disrupted upon conversion of the AS phase to the HS or HF phase has yet to be established experimentally, although it appears that lipid-water hydrogen bonding is extensive in the HF phase in particular. In contrast to the AS to HF transition, the transition of the metastable, but long lived HS phase to the HF phase at 323.3 K exhibits a considerably lower latent heat. One implication is that the hydrogen bonding makes almost no contribution to the latent heat of the transition between the hydrated phases. One of the principle results of the present study is to confirm the above hydrogen bond picture for a model of PE lipid bilayers.

There have been a number of models relevant to hydrogen bonding in lipid bilayers. The earlier models were phenomenological models for the effect of hydrogen bonding on the transition temperature of the main phase transition proposed by Nagle [12] and Eibl and Wooley [13]. Recently, Zhang *et al.* [14] studied a model for a hydration-dehydration phase transition involving hydrogen bonds between PE polar heads where each molecule can have two hydrogen bond donors at right angles, and two acceptors antiparallel to the donors. This model will be incorporated into the present study and will be described in detail below. A related model is that studied in considerable detail by MacDonald, Pink, and Quinn (MPQ) [15, 16]. Their model applies to those hydrated ceribrosides in which there is a single donor-single acceptor complex on the amide group giving rise to a "striped" ground state composed of $1d$ hydrogen bonding networks. This model included both hydrogen bonding effects and chain melting at the main phase transition. Two earlier models also included a site degree of freedom which cannot bond with neighboring sites, and other degrees of freedom which are able to, but need not, bond with neighboring sites. They are a Potts lattice gas model of krypton adsorbed on graphite proposed by Berker, Ostlund, and Putnam [17], and a site-bond percolation model, with temperature dependent bond probability proposed by Coniglio, Stanley, and Klein [18] to study the sol-gel transition. However, in both of the latter two models the bonds are not correlated with the relative position of the sites. This is a major difference between the latter two models, and the models studied by Zhang *et al.* and MPQ.

The organization of the rest of this paper is as follows. In Sec. II we describe in detail our model which accounts for both the chain conformations and the hydrogen bonding of the polar heads. In Sec. III we describe the Monte Carlo method we used including a subtle issue regarding detailed balance. In Sec. IV we describe our equilibrium results for the three phases transitions: chain melting transition of DMPC, HS to HF transition for DMPE, and the AS to HF transition of DMPE. Finally, in the last section we provide an overall summary and suggest further work to be done.

II. PHENOMENOLOGICAL LATTICE MODEL FOR DMPE

In this section we give a detailed description of the model used to describe DMPE. The model combines the multistate model due to Pink, Green, and Chapman (PGC) which describes the chain states with our own hydrogen bonding (HB) model [14] which describes the hydrogen bonding states of the polar heads.

The PGC model accounts for the acyl-chain conformational statistics and the interchain van der Waals interactions. Each tail or chain is placed on a site of a triangular lattice. The lattice serves to partially account for the excluded volume effect. Since each lipid molecule has two chains, two neighboring sites on the lattice represent a single molecule. This will become important when we consider the HB states. The chain conformations are represented by ten single chain states labeled $\alpha = 1, \ldots, 10$. Each state is described by a cross-sectional area A_α, a chain length d_α, an internal energy ϵ_α, and an internal degeneracy D_α. The volume $A_\alpha d_\alpha$ of each chain conformation is independent of the particular conformational state. The values of these parameters for DMPC or DMPE are shown in Table I. The first state is the all-*trans* ground state. The next eight states are low lying conformational excitations of the ground state. These nine states are characteristic of the gel phase. The tenth state is a high energy state which is a combination of many disordered chain conformations and which is thus characteristic of the fluid phase. The Hamiltonian for the PGC model can be written as

$$\mathcal{H} = \sum_i \sum_{\alpha=1}^{10} (\epsilon_\alpha + \Pi A_\alpha)\mathcal{L}_{\alpha,i} - \frac{J_0}{2} \sum_{\langle i,j \rangle} \sum_{\alpha,\beta=1}^{10} I_\alpha I_\beta \mathcal{L}_{\alpha,i}\mathcal{L}_{\beta,j} \tag{1}$$

TABLE I. Single chain parameters for ten-state model for chains of 14 monomers appropriate for DMPC and DMPE.

Chain state	α	A_α (Å^2)	d_α (Å)	ϵ_α (10^{-13} ergs)	D_α
all-*trans*	1	20.40	16.25	0	1
Jog	2	22.10	15.00	0.45	4
	3	24.11	13.75	0.45	4
	4	26.52	12.50	0.45	4
Kink	5	22.10	15.00	0.90	16
	6	24.11	13.75	0.90	12
	7	26.52	12.50	0.90	8
	8	24.11	13.75	1.35	48
	9	26.52	12.50	1.35	64
Fluid	10	34.00	9.75	1.94	39,366

where $\langle i,j\rangle$ are labels for nearest neighbor sites and $\mathcal{L}_{\alpha,i}$ is an occupation variable which equals 0 unless site i is in state α.

The effective lateral pressure Π should not be thought of as a lateral tension (in the sense of a surface tension). It was introduced by Marcelja [19] to describe all the interactions other than the van der Waals interactions between lipid-acyl chains. The lateral pressure can therefore be considered as an effective lateral pressure which corresponds to interactions involving the polar heads plus the hydrophobic effect. The value of Π and the strength of the van der Waals interaction J_0 is determined by a fit to the transition temperature and enthalpy of DMPC. The factor I_α accounts for the variation in the van der Waals interaction with distance and chain conformation, and is given by $I_\alpha = (1.8\,A_1/A_\alpha - 0.8)(A_1/A_\alpha)^{5/4}$. For the fluid state this form for I_α does not hold, and the interaction strength is reduced by a "weakening factor" equal to 0.4.

Corvera *et al.* [20] showed by computer simulation that the PGC model does not predict a first order phase transition for the parameters describing DMPC, dipalmitoyl phosphatidylcholine (DPPC), and distearoyl phosphatidylcholine (DSPC) bilayers. Zhang *et al.* [21] then showed that an additional term in the Hamiltonian due to the hydrophobic mismatch between chain conformations of different length gave rise to a first order transition for DPPC. This term is given by the following mismatch Hamiltonian:

$$\mathcal{H}_{mis} = \frac{\gamma_{mis}}{2} \sum_{\langle ij\rangle} \sum_{\alpha\beta} |d_\alpha - d_\beta| \mathcal{L}_{\alpha,i} \mathcal{L}_{\beta,j} \tag{2}$$

with $\gamma_{mis} = 5 \times 10^{-16}$ ergs/Å for DPPC.

The PGC model has been very successful in interpreting a variety of data relating to phase behavior for pure PC bilayers [22], lipid-cholesterol bilayers [23], mixed lipid bilayers [24], and lipid-protein bilayers [25], and in predicting a variety of physical properties. The advantage of the model in conjunction with the Metropolis Monte Carlo simulation method is that it can be used to understand phenomena for length scales much larger than intermolecular distances, since a considerably larger system can be simulated using the simple PGC model than using a full molecular model. This has proved particularly useful for studies of phase transitions and phase separation phenomena.

We now want to include the effect of hydrogen bonding between polar heads which is essential for an understanding of DMPE. In a previous work [14], a model of hydrogen bonding was developed for a square lattice where each lattice site represented a PE polar head which can have a maximum of four possible hydrogen bonds (HB's) with the polar heads of neighboring molecules. Each polar head has two HB donors and two HB acceptors. The structure of the polar head is such that the two donors are perpendicular to each other and the donors are antiparallel to the acceptors. In the model each lattice site can be in one of five possible HB states. Four of these states are called bonding states, and such states consist of two vectors at right angles representing the direction of the hydrogen bond donors. Two vectors representing the acceptors then point in the opposite directions to the donors. If a bond between a donor and acceptor exists it is assumed to lie along a link in the square lattice. There are four unique orientations in which these two vectors can point along the links of the lattice, and these four unique orientations correspond to the four bonding states. A hydrogen bond between neighboring sites i and j is formed if the donor vector of i points toward j and an acceptor vector of j points towards i, or an acceptor vector of i points toward j and a donor vector of j points toward i. If such a bond is formed, the energy is lowered by an amount E_b. In addition there is a fifth state, called the unbonding state, which cannot form hydrogen bonds with any of its neighbors. This state has a degeneracy D_u associated with it. The degeneracy accounts for the many orientations of the polar head that cannot bond with their neighbors as well as the possibility of hydrogen bonding of water molecules to the polar heads competing with the hydrogen bonding between the polar heads.

We have adapted this hydrogen bonding model so that it can be included with the PGC model for the chain states. We consider that two neighboring chain sites on the triangular lattice represent a single lipid molecule (see Fig. 1). If both sites are in the all-*trans* lowest energy state then the lipid represented by these two sites can hydrogen bond to a neighboring lipid. If the two polar heads are too far from each other, then hydrogen bonding cannot occur. This would occur, for instance, if the chains were in excited states. Thus, if either chain of a lipid is in an excited state then that lipid cannot hydrogen bond to its neighbors. The system of lipid molecules forms a rectangular lattice as shown in Fig. 1, and we can treat this lattice in the same way as the original square lattice for the HB model. The polar heads are fixed in position; however, this is acceptable because the hydrogen bonding is only important in the AS phase. The hydrogen bond energy E_b and the unbonding state degeneracy

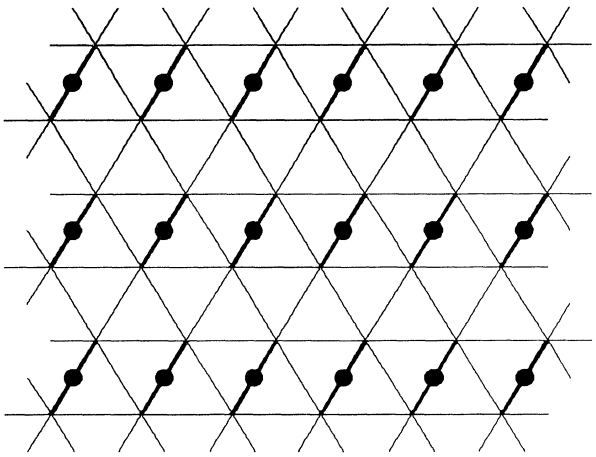

FIG. 1. Lattice used to model anhydrous DMPE. The sites of the triangular lattice represent lipid chains, and the sites of the rectangular lattice represented by filled disks represent the polar heads. The two chain sites and the polar head connected by a heavy line comprise a single lipid molecule.

D_u can be adjusted so that the transition temperature for anhydrous DMPE is approximately equal to that found experimentally.

The Hamiltonian for hydrogen bonding can be written as

$$\mathcal{H}_{HB} = -E_b \sum_i f(\vec{S}^{(x)}_{\vec{r}_i} \cdot \vec{S}^{(x)}_{\vec{r}_i+\hat{x}}) + f(\vec{S}^{(y)}_{\vec{r}_i} \cdot \vec{S}^{(y)}_{\vec{r}_i+\hat{y}}), \qquad (3)$$

where $f(1) = 1$, $f(-1) = f(0) = 0$, and $\vec{S}^{(x)}_{\vec{r}_i}$ is a horizontal unit vector and $\vec{S}^{(y)}_{\vec{r}_i}$ is a vertical unit vector pointing in the direction of the HB donors and located at sites on a rectangular lattice denoted by $\vec{r}_i$. For the unbonding state both these vectors are taken to be 0.

III. CALCULATIONAL TECHNIQUES

We use a standard Metropolis Monte Carlo method adapted to states with degeneracies. Mouritsen [22] has shown how degeneracies D such as D_u and D_α should be treated in Metropolis Monte Carlo simulations as follows. One chooses a trial different state with equal probability from the remaining states (i.e., not including the old state), and then one replaces the usual change in energy ΔE by $\Delta E - k_B T \Delta(\ln D)$ in the computation of the Boltzmann factor.

Now we discuss how to make trial moves. Updates of the chain states are made separately from updates of the HB states. However, there is an interdependence between the two kinds of updates because only lipids whose chains are both in the all-*trans* state can hydrogen bond with other lipids. First we discuss the chain updates. A chain is chosen at random and a trial new state, different from the old state, is chosen at random. If the old state of this chain and the state of the other chain in the same lipid molecule are both in the all-*trans* state, then the new chain state will cause the polar head to break any hydrogen bonds it has with neighboring lipid molecules. When this happens we include in the Boltzmann factor the energy of lost hydrogen bonds. If this trial new state is accepted according to the Metropolis algorithm, then we change the chain state and keep track of the HB state the polar head had before the trial move was accepted. Saving the HB state is necessary to preserve detailed balance, as we will discuss below. If the new trial state of a chain and the chain state of the other chain in the same lipid molecule are both in the all-*trans* state, we then use the same HB state associated with the polar head when both chains were last in the all-*trans* state. In this case the new chain state can cause the creation of hydrogen bonds with the neighboring lipid molecules, and the decrease in the energy resulting from the formation of these hydrogen bonds is included in ΔE when computing the Boltzmann factor. If neither of the above two cases occurs then the hydrogen bond states are ignored.

To see why it is necessary to save the last HB state of a lipid molecule, consider the following scenario for a simulation of anhydrous DMPE. At low temperatures most of the chains are in the all-*trans* state and most of the polar heads are hydrogen bonded with their neighbors. With hydrogen bonding (anhydrous DMPE) the transition is at approximately 330 K, while without hydrogen bonding (hydrated DMPE) the transition is approximately 324 K. Consider a temperature between these two transition temperatures, which corresponds to the low temperature state for anhydrous DMPE, but the high temperature state for hydrated DMPE (where most of the chains are in the high energy random fluidlike conformation denoted as the tenth state). Suppose a fluctuation causes the chain state to change. Since this excitation costs energy, we would expect that rather quickly the chain state will fluctuate back to the original all-*trans* state. However, if we do not include the previous HB state of the polar head, then the Boltzmann factor will only include the energetics of the chain states, and the probability that the chain state will revert back to its all-*trans* state is very low. From the point of view of this chain, the system is the hydrated DMPE rather than the anhydrous DMPE we wish to simulate. The reason for this failure is the lack of detailed balance. Detailed balance, which is necessary to insure that the system is able to reach equilibrium, requires that $P(i \to j)\exp(-\beta E_i) = P(j \to i)\exp(-\beta E_j)$. Here $P(i \to j)$ is the transition probability from state i with energy E_i to state j with energy E_j. If we do not save the HB states then only the breaking of hydrogen bonds will be included in the Boltzmann factors and detailed balance will be violated by a large factor of order $\exp(-m\beta E_b)$, where m is the number of broken hydrogen bonds.

When making Monte Carlo moves for the HB states we, of course, only consider pairs of sites which are both in the all-*trans* state and neighboring lipids whose chains are also both in the all-*trans* state. Here detailed balance is not violated since changing the HB states does not require the chain states to change. An important issue is whether the chain states or the HB states should be updated more often. *A priori* we do not have any knowledge of the time scales for fluctuations for the two kinds of states. However, at the transition the energetics of the chain states are comparable to that of the HB states. Thus, we do an equal number of chain updates as head updates. For the anhydrous DMPE simulation we find that very few new chain or HB states are accepted below the transition temperature except very close to the transition temperature. Above the transition there is essentially no hydrogen bonding and the MC acceptance rate is 10–20 %.

One of the key quantities of interest from our simulations are the latent heats for the various transitions. To obtain these latent heats it is essential to determine accurately the location of the transition and whether or not it is first order. In some cases the jump in the energy at a particular energy is so strong that it is obvious that the transition is first order and the latent heat can be estimated from the jump in energy. For a true first order transition the specific heat C will exhibit a δ function peak in the thermodynamic limit, which will show up as a sharp peak for large but finite size systems. The latent heat can be estimated from $\int C dT$, where the integral is over a narrow range of temperatures around the transition. However, as Zhang *et al.* [21] have shown a first or-

der transition was absent in some previous simulations of DMPC, DPPC, and DSPC. As stated above, they added the mismatch interaction between the chains and were able to show using the Lee-Kosterlitz (LK) method [26, 27] that the resulting transition was first order. The Lee-Kosterlitz method begins with a free energylike quantity

$$F(X,L,T) = -\ln P(X,L,T), \tag{4}$$

where X is either the total internal energy E or the total area A. L is the linear dimension of the system, T is the temperature, and $P(X,L,T)$ is the probability of the observable X occurring. Note that for the PGC model the total area is the sum of the cross-sectional areas of the chains, and can be considered as a type of order parameter. A first order transition can be driven by either varying the pressure or the temperature. In the former case one examines $F(A,L,T)$ in the latter case $F(E,L,T)$. In the PGC model the transition can be driven in either way. We choose to work with $F(E,L,T)$ because it is easier to compute. At a phase transition at temperature T_m, $F(E,L,T_m)$ will have two equal minima at E_1^{min} and E_2^{min} corresponding to the energies of the low and high temperature phases, respectively. These two minima will be separated by a free energy maximum at E^{max} corresponding to a domain wall between the two phases. In the bulk limit $L \to \infty$, the quantity $F(E,L,T_m)L^{-d}$ will be independent of E for $E_1^{min} \le E \le E_2^{min}$. However, for finite L, an expansion of $F(E,L,T_m)$ will have a double-minima structure of the form

$$F(E,L,T_m) = L^d F_0(E,T_m) + L^{d-1}F_1(E,T_m) + \cdots, \tag{5}$$

where F_0 is the bulk free energy density which is constant for $E_1^{min} \le E \le E_2^{min}$. Thus, to first order

$$\Delta F(L) \equiv F(E^{max},L,T_m) - F(E^{min},L,T_m) \sim L^{d-1}. \tag{6}$$

If the transition is second order then $\Delta F(L)$ is independent of L, and if there is no transition then $\Delta F(L)$ will decrease with L.

To implement the LK analysis one calculates the probability $P(E,L,T)$ for many temperatures near T_m, and defines T_m as the temperature where the free energy at the two minima are equal. This is most effectively accomplished using the Ferrenberg-Swendson [28] (FS) histogram method where one runs a long simulation at an inverse temperature β_{sim}, and determines the probabilities at $\beta = 1/T$ by

$$P(E,L,T) = h(E)e^{-(\beta-\beta_{sim})E}, \tag{7}$$

where $h(E)$ is the fraction of the configurations with total energy E.

Our simulations were carried out on lattices of size 4×4 to 40×40 with most of the work done at 20×20. Typically, at each temperature 10 000 Monte Carlo steps per site (MCSS) were used for equilibration and another 10 000 for averaging. When using the LK analysis typically we annealed our system from low temperatures at a rate of 10^{-4} degrees/MCSS, and computed $h(E)$ for at least 10^5 MCSS.

IV. EQUILIBRIUM RESULTS

We first set out to duplicate the results for DMPC found in the literature [29, 30]. Using experimental data the pressure for DMPC is $\Pi = 30$ dyn/cm and the interaction strength $J_0 = 0.618 \times 10^{-13}$ ergs [29]. Initially, we used the same value for the mismatch parameter $\gamma_{mis} = 0.005$ (in units of 10^{-13} ergs) as was used for DPPC [21]. Previous work has shown that the mismatch interaction term does not change the thermodynamic data such as the transition temperature and latent heat significantly. Its main effect is to induce a weak first order transition where one was previously absent. In addition one expects that γ_{mis} might be different for DMPC and DMPE. Our results are consistent with previous work on DMPC. However we found using the Lee-Kosterlitz analysis that there was only a crossover from the low temperature to the high temperature phase. We then increased γ_{mis} until a first order transition was obtained. This occurred at about $\gamma_{mis} = 0.007$. Results using the FS histogram method are shown in Fig. 2 for 10^6

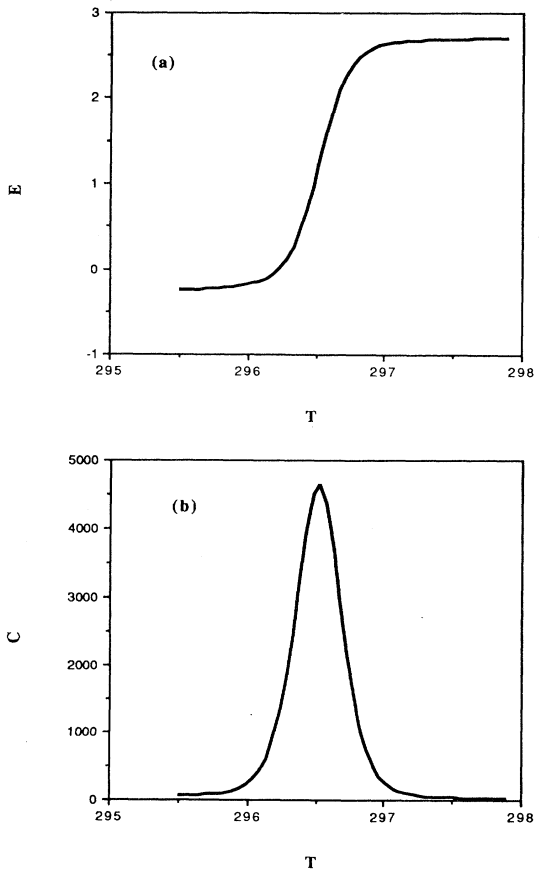

FIG. 2. DMPC results using the FS histogram method for 10^6 MCSS on a 20×20 lattice simulated at 296.7° with $\gamma_{mis} = 0.007$. (a) Internal energy in units of 10^{-13} ergs versus temperature in degrees Kelvin, (b) specific heat in units of 10^{-13} ergs per degree Kelvin versus temperature.

MCSS on a 20×20 lattice simulated at 296.7°. Using the LK analysis, we estimate the transition temperature is at approximately 296.6 ± 0.2 K which is consistent with the experimental value. The latent heat per lipid molecule is difficult to measure since the transition is not strongly first order. The area under the specific heat curve gives an upper bound to the latent heat of about 8.1 kcal/mol. A lower bound can be obtained by considering only the area under the specific heat peak between the temperatures where the specific heat reaches half its maximum. This estimate gives a latent heat of 5.8 kcal/mol. The true value for this model is probably somewhere in between. Thus, we estimate the latent heat at 6.9 ± 0.6 kcal/mol which compares favorably with the experimental value of 6.7 kcal/mol [31] and a previous value from numerical simulation of 7.2 kcal/mol [30].

As we have seen the chain melting transition is not strongly first order. The microscopic configurations resemble in many ways those found in second order transitions near the critical point, i.e., there are large fluctuations. We have examined in detail the configurations for a 20×20 lattice near the transition (see Fig. 3). At 1° below the transition approximately 57% of the chains are in the all-*trans* state, 35% are in the excited states, and 8% are in the fluid state. Fluid droplets reach a size of approximately ten chains, with occasional occurrence of larger droplets. The excited states also tend to form domains separate from chains in the all-*trans* state. At the transition the sizes of fluid droplets fluctuate rapidly with as many as half the chains in the fluid state. At 1° above the transition the percentages of the various states changes sharply, with about 3% in the all-*trans* state, 7% in the excited states, and 90% in the fluid state. Many of the chains in the low energy states are clustered together into a few separate domains.

Next we wish to simulate the HS to HF transition of hydrated DMPE. The hydrated phase of DMPE prevents hydrogen bonding between the polar heads, and thus its behavior is similar to DMPC at a higher pressure. We thus adjusted the pressure in our simulations until the transition temperature was approximately equal to that found experimentally, 323.3 K. The pressure necessary to do this is approximately $\Pi = 55$ dyn/cm. All other parameters were kept constant. The results of this simulation showed a gradual crossover between the low temperature all-*trans* state and the fluid state. The results are independent of heating and cooling (no hysteresis), which suggests that there is no transition for this system. The lack of a transition was confirmed using the LK analysis. We thus increased the mismatch parameter to the minimum value necessary to obtain a first order transition, which we found to be about $\gamma_{mis} = 0.012$. Figure 4 shows the energy versus temperature and specific heat using the FS histogram method with 10^6 MCSS on a 20×20 lattice. Our estimate of the transition temperature is 322.5 ± 0.2 K with a latent heat of 6.5 ± 0.6 kcal/mol, which is consistent with the experimental value of 6.1 ± 0.6 kcal/mol [1]. The nature of the configurations near the chain melting transition for hydrated DMPE is similar to that of the DMPC.

The transition for the anhydrous DMPE to hydrated fluid DMPE is much different from what we described above for DMPC and hydrated DMPE. The transition is very strongly first order. The low temperature phase is almost entirely composed of chains in the all-*trans* state and polar heads strongly hydrogen bonded with their neighbors. There are very few fluctuations to excited

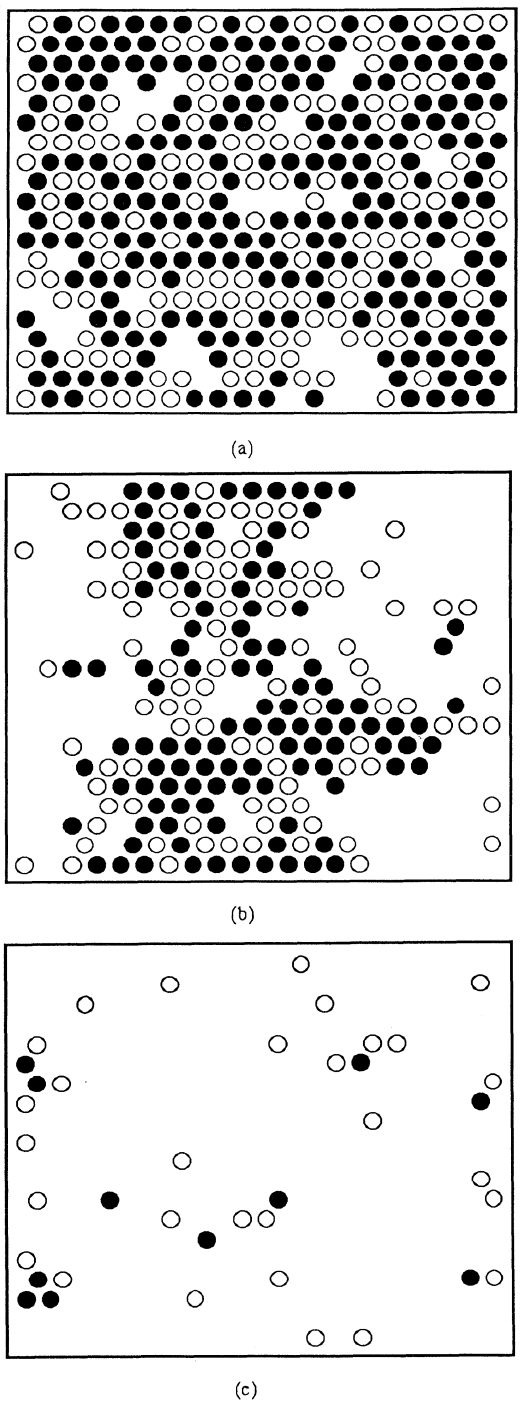

FIG. 3. Microscopic configurations for DMPC. Filled circles correspond to the all-*trans* state, open circles to the excited states, and positions with no circle to the fluid state. (a) $T = 295.6$ K , (b) $T = 296.6$ K, and (c) $T = 297.6$ K.

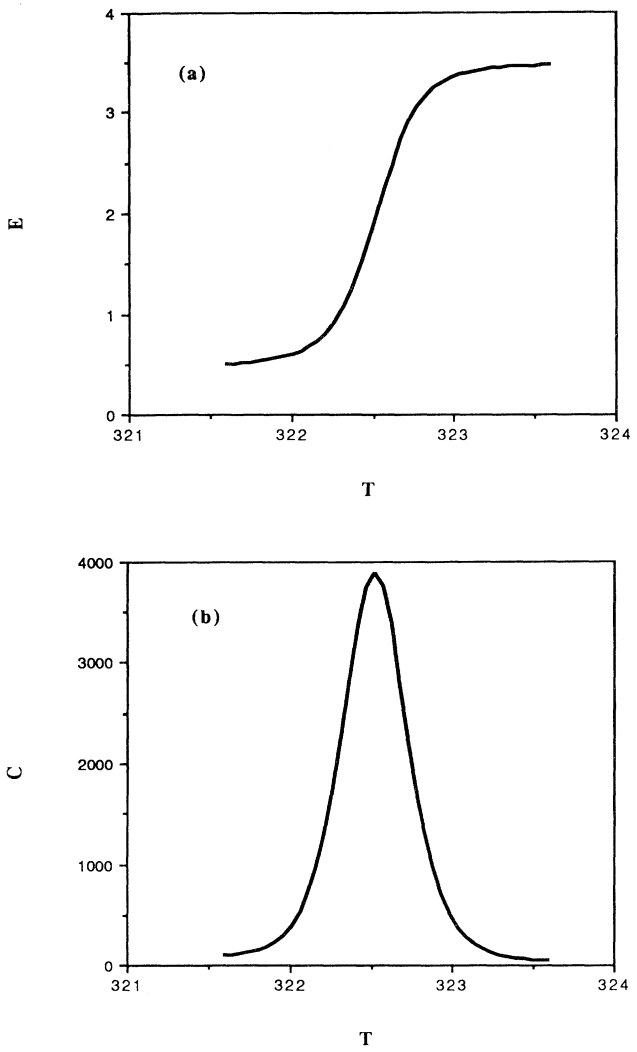

FIG. 4. Hydrated DMPE results using the FS histogram method for 10^6 MCSS on a 20×20 lattice simulated at 322.6° with $\gamma_{mis} = 0.012$. (a) Internal energy versus temperature, (b) specific heat versus temperature. Units are the same as in Fig. 2.

states until the temperature is within about a tenth of a degree of the transition. Once the transition occurs, the hydrogen bonds disappear rapidly and the chains melt to the fluid states. We used the same parameters for the chain Hamiltonian as was used for hydrated DMPE. To obtain a transition temperature near that found experimentally (between 330 K and 331 K), we used a nonbonding state with degeneracy $D_u = 40\,000$ and found that $E_b = 2.2$ in units of 10^{-13} ergs. The latent heat is easy to measure from the jump in the internal energy and was given by 18.8 ± 0.2 kcal/mol which compares very well with the experimental value of 19.5 ± 1.9 kcal/mol [1]. The transition is clearly driven by the hydrogen bonding, since the following simple estimate of the transition is very close to the actual transition. We estimate the transition as the point where the free energy of the unbonding state equals that for two hydrogen bonds, i.e., T_m occurs when $2E_b - T_m \ln D_u = 0$. With $T_m = 330.5$ K and $D_u = 40\,000$, one finds $E_b = 2.4$, which is very close to that found in the simulations. If we change D_u to 4000 then our simulations show that we should choose $E_b = 1.87$ which again is very close to the simple estimate of $E_b = 1.9$. The latent heat then decreases to 17.9 ± 0.2 kcal/mol.

Microscopically, the hydrogen bonds are so strong that the chains are kept in the all-*trans* state until the temperature is high enough to break a hydrogen bond. When the hydrogen bonds disappear, then the system is equivalent to the hydrated DMPE about 10° above the chain melting transition for hydrated DMPE. Thus, as soon as the hydrogen bonds are broken the chains very quickly occupy the fluid chain state. Because one must have four neighboring chains (two in each lipid) in the all-*trans* state to form a hydrogen bond, it is very difficult to recreate the hydrogen bond network by cooling the system. Thus, there is very strong hysteresis, and the transition on cooling is at a temperature much lower than 330 K. In addition our estimate for the transition from the AS to HF phase given above is difficult to reproduce by slowly heating the system from the low temperature phase, since the system tends to display superheating. Thus, we have developed another technique for determining the transition temperature. We begin with a lattice entirely bonded, and then set the chain state in a 4×4 piece of the lattice to the fluid state. This represents a fluctuation which created a HF domain coexisting with the AS phase for the rest of the lattice. We then run the simulation at various temperatures near the transition, and determine whether the system melts to the HF phase or eliminates the HF domain and remains in the AS phase. We repeat this procedure for about five different sets of random number seeds. Our results show that to obtain a transition temperature near the experimental value we need $E_b = 2.35$ for $D_u = 40\,000$. This is slightly higher than our previous estimate. With this value we find a transition temperature $T_m = 330.3$ K and a latent heat of 19.2 ± 0.2 kcal/mol.

The values of D_u are high enough to give a sharp first order phase transition (Zhang *et al.* found first order transitions for the HB model without chain degrees of freedom for $D - u > 160$ [14]. We expect D_u to be high since it includes a continuum of unbonding states induced by competitive hydrogen bonding by the polar head with water molecules. This work has led to the possibility of the investigation of this transition using NMR techniques and this should enable us to get a better understanding of E_b and D_u as we can predict the first moment of the quadrupolar NMR spectrum from our results.

V. CONCLUSION

In this work we have proposed and analyzed a model for lipid bilayers which includes both hydrogen bonding and the interaction between hydrocarbon chains. This is a natural extension of our previous work on hydrogen bonding in PE bilayers [14]. We have been able to determine the parameters necessary to produce phase transitions in our model at approximately the same tem-

peratures as in experimental work for the chain melting transition of DMPC, hydrated DMPE, and anhydrous DMPE. Our results for the latent heats for all three systems compare favorably with those obtained experimentally. This provides strong evidence that hydrogen bonding is the primary cause for the difference between the transition temperatures in DMPC and DMPE.

We also have developed a procedure for incorporating two sets of microscopic states (chain states and HB states) which are not independent. In our method we save the HB states even though they have no effect on the energy when the chains are not in the all-*trans* state. Our procedure might be relevant to other models with similar microscopics. For example, consider a site-bond model of gelation where there is an energy associated with a bond between two occupied sites as well as an Ising-like interaction between sites. Here a bond could only occur when the sites are occupied. If two sites are connected by a bond and one site changes to unoccupied, then the bond is also destroyed. A simulation of such a model would require a procedure analogous to ours in order to preserve detailed balance.

Some of the results reported here were obtained by using the extrapolation method of Ferrenberg and Swendsen [28]. The nature of the phase transitions was firmly established by means of the finite size scaling method of Lee and Kosterlitz [26, 27]. In particular, we were able to determine that the size of the mismatch parameter γ_{mis} used for DPPC is insufficient to produce a first order phase transition in DMPC. The small difference in γ_{mis} between DPPC and DMPC may be due to the inaccuracy in determining the parameters for DMPC in previous work [29, 30]. The transition in DMPC is much weaker than that in DPPC as observed in the experiments. Therefore the mean-field theory, used in Ref. [30], is not expected to work very well.

We also found that γ_{mis} depends on the nature of the polar heads. Since the mismatch interaction originates from the interaction of water molecules with the lipid chains and since the nature of the polar heads may affect the structure and density of water at the microscopic level, we would expect the mismatch interaction constant to differ for DMPC and DMPE. This difference should correlate strongly with the pressure, which describes the effect of the polar heads in the original PGC models. Our work suggests that, approximately, the minimum value of γ_{mis} necessary for a first order transition is proportional to the pressure.

In this study we have developed a satisfactory model of DMPC and DMPE bilayers. We are currently working on simulations of mixtures of the two kinds of lipids. The focus of our work so far has been only on the hydrogen bond effects. Future work will add in the effects of the chain states, as well as including cholesterol as an impurity. In addition we are exploring the possibility of using our HB model to investigate the effects of hydrogen bonding on the thermodynamic behavior of water, which has been recently investigated in a mean field approach [32].

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