Simulation of the Gel–Fluid Transition in a Membrane Composed of Lipids with Two Connected Acyl Chains: Application of a Dimer-Move Step

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ABSTRACT Phospholipids have been treated as dimers on a hexagonal lattice, and a move has been introduced that allows the dimers to move and change their orientation on the lattice. Simulations have been performed in which phospholipid chains have been treated as being either independent or infinitely coupled thermodynamically with regard to their conformational state. Both types of simulation have reproduced well experimental heat-capacity curves of dipalmitoyl phosphatidylcholine small unilamellar vesicles. Apart from a different gel–fluid interaction parameter and a different number of unlike nearest-neighbor contacts, most of the averages and thermodynamic quantities were essentially the same in the two types of simulation. These results indicate that the transition is not first order and validate those of previous Monte Carlo simulations that have neglected the dimeric nature of phospholipids in the sense that they show that for the thermotropic transition the approximation of phospholipids as monomers is valid.

INTRODUCTION

Biological membranes are constituted by a lipid matrix in which integral proteins are embedded and to which peripheral proteins are adsorbed (Singer and Nicholson, 1972). This lipid matrix is a quasi-two-dimensional bilayer, which owes its structure (Cevc and Marsh, 1978) to the amphipathic nature of the phospholipid molecules that constitute it and orient themselves in opposite directions in the two leaflets of the bilayer in such a way that the hydrophilic parts of the lipids face the aqueous external medium and the hydrophobic parts, the acyl chains, form the nonpolar interior of the bilayer. The acyl chains of the phospholipids can exist in a state in which the rotational conformers about the carbon–carbon bonds are predominantly *trans*, or in a state in which an appreciable number of *gauche* conformers exist (Nagle, 1980). Bilayers made of pure lipids that have their acyl chains in an all-*trans* conformation show a high degree of long-range order in the plane of the bilayer and small diffusion coefficients; at very low temperatures they form a crystal, and at somewhat higher temperatures they form a gel. At still higher temperatures the acyl chains of the phospholipids become disordered and the bilayer undergoes a transition to a fluid state, in which the diffusion coefficient in the plane of the membrane is large.

In the present study we are concerned with the thermotropic transition between the gel and the fluid states of the lipid membrane. We simulate this membrane as a triangular lattice (each site has six nearest neighbors) in the two dimensions representing the plane of a bilayer. We use the Monte Carlo method (Metropolis et al., 1953; Binder, 1979) to obtain average thermodynamic properties of interest. This simulation technique has been applied to lipid membranes quite extensively (Mouritsen, 1990, 1991), with a 10-state model (Pink et al., 1980) used for each lipid chain. Recently Sugar et al. (1994) used a two-state model (Doniach, 1978; Georgallas and Pink, 1982) in which each lipid molecule is assumed to exist in either the gel or the fluid state, with no intermediate states allowed. This simpler model, which falls under the general category of the Ising model (Ising, 1925; Onsager, 1944), was able to reproduce the essential characteristics of the gel–fluid phase transition in small unilamellar vesicles of phosphatidylcholine. Still using a two-state model and similar Monte Carlo tools, we address here the following problem.

In lipid bilayers in the crystal, gel, and fluid states, the acyl chains of the phospholipids occupy the sites of a triangular lattice (the coordination number is 6) in the plane of the bilayer (Ruocco and Shipley, 1982), though the lattice spacings are different in each case. In attempts to perform a Monte Carlo simulation of a phospholipid membrane on a lattice, two alternative options have generally been envisaged. The first is to consider that the acyl chains occupy the lattice sites and behave independently of each other; this provides the correct lattice geometry but ignores covalent connection and thermodynamic coupling between the chains (Mouritsen, 1990). The second is to consider that each lipid occupies one lattice site and behaves as a single unit (Sugar et al., 1994); thermodynamically, this means that the two chains are infinitely coupled so that each chain is in the same configurational state. This is physically more correct for each lipid but leads to an incorrect lattice geometry, because it is the acyl chains, and not the lipids as a whole, that occupy sites having a coordination number of 6 (Ruocco and Shipley, 1982).

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In all previous studies using the Monte Carlo method, including a recent study from this laboratory (Sugar et al., 1994), lattice points have been allowed to alter their state, governed only by the energetics of the transition. Whether each lattice point is taken to represent an acyl chain or an entire phospholipid molecule is a matter of interpretation of the simulation results (the main transition enthalpy to be used differs, of course, by a factor of 2 in the two cases) and leads to somewhat different gel–fluid interaction Gibbs free energies. The question we now ask is: How significant is the approximation involved in these treatments?

The goal of this research is to assess the importance, in Monte Carlo simulations of lipid membranes, of taking (or not) into account the fact that phospholipids possess two covalently connected and thermodynamically coupled acyl chains. Here, we assume that the acyl chains of the lipids occupy the sites of a triangular lattice and that pairs of chains (lipid molecules) are permanently connected, forming dimers on the lattice. The problem that arises is the following: If, in the Monte Carlo calculations (Binder, 1979), we use only Glauber steps (in which, in each cycle, we ask the question of whether a chain at a given site will change state) and Kawasaki steps (in which, in each cycle, we ask the question of whether a molecule will interchange positions with one of its neighbors) the orientation of the dimers on the lattice is fixed by the initial configuration, because individual chains will not be interchanged. A new type of step was therefore introduced that allows for the sampling of the different orientational configurations of the dimers on the lattice.

In this paper we compare the results obtained for the heat-capacity function, the cluster-distribution function, and the percolation probability with three different sets of assumptions: i) the sites of the triangular lattice are occupied by individual and independent chains, ii) the lattice sites are occupied by individual chains that are infinitely coupled, so that the chains of one lipid are both in the same conformational state, and iii) the lattice sites are occupied by lipid molecules in which the chains are infinitely coupled. We conclude that no major differences arise among the simulations using the various different sets of assumptions.

METHODS

Lattice model with dimers

We consider that each lipid chain can exist in either of two states: gel (ordered) or fluid (disordered), characterized by a given set of state functions (Gibbs free energy $\mathcal{G}$, enthalpy $\mathcal{H}$, and entropy $\mathcal{S}$, subscripts g being used for the gel state and l for the liquid-crystalline or fluid state). Each chain interacts only with its nearest neighbors in the lattice; this interaction is described by a free energy ϵ_{ll} for a fluid–fluid contact, ϵ_{gg} for a gel–gel contact, and ϵ_{gl} for a gel–fluid contact. In the case of coupled, connected chains the states of the two elements of the dimer are assumed to be infinitely coupled; that is, the molecule must be in either the gel or the fluid state but cannot have one of its chains in each state.

The first step in the algorithm is to fill the lattice with dimers in a random orientation. If on a certain site no dimer can be positioned because of restrictions that are due to dimers already present on the lattice, a small number of dimers is removed and the process is repeated until the lattice is completely filled.

The Monte Carlo method (Binder, 1979) is based on sampling the configurational space of the system. In general, by configuration we mean a lattice for which the number and the position of gel and fluid chains are specified. In the present study the elements of each dimer are also specified. To meet the requirement of complete sampling we introduce a new step, a dimer move, in which two dimers participate (Fig. 1), and use alternating Monte Carlo cycles of Glauber steps (Binder, 1979) and dimer move steps.

In the Glauber step cycle each site (i, j) in the lattice (or an equivalent number of randomly picked sites in the lattice) is visited and the probability of changing the state of the individual lipid is calculated. This probability (Metropolis et al., 1953) is given by the Boltzmann factor

$$P_{ij} = \exp(-\delta G/RT), \tag{1}$$

where δG is the Gibbs free-energy difference between the initial configuration and one in which the state of the lipid under consideration has been changed (see Eq. 12 below). If $\delta G \leq 0$ the change is always accepted; otherwise, if $\delta G > 0$, a random number is drawn and the change is accepted if

$$\mathrm{RAN} \leq P_{ij}, \tag{2}$$

otherwise it is rejected, and we proceed to another lattice site.

In the dimer move step cycle, each site is visited and the possibility of a concerted movement involving the lipid under examination and any one of its neighbors is assessed. Note that not all neighbors are susceptible to participating in the dimer move; the two lipids involved (four acyl chains) must be side by side, in a position equivalent to that shown in Fig. 1. The probability that any selected lipid has a neighbor susceptible to participate in a concerted move is 0.75 on a randomly filled lattice. If the move is possible, the probability that it occurs is calculated in a manner analogous to that used for the Glauber step. If more than one neighboring lipid can exchange, the one for which a change is attempted is selected randomly.

Evaluation of thermodynamic functions

The connection between the simulation and experimental data is made through the comparison of calculated and experimental excess heat-capacity functions. We calculate the excess heat-capacity function by using the fluctuation-dissipation theorem (Hill, 1960) according to the following scheme. The Gibbs free energy G of the system (excluding the configurational entropy term) is given by

$$G = n_g\mathcal{G}_g + n_l\mathcal{G}_l + n_{gg}\epsilon_{gg} + n_{ll}\epsilon_{ll} + n_{gl}\epsilon_{gl}, \tag{3}$$

where the subscripts g and l refer to gel and fluid, respectively, the double subscripts refer to contacts, n is the number of occurrences of each type, and script letters refer to state functions per site. In a triangular lattice

$$n_{gg} = \frac{zn_g - n_{gl}}{2}, \tag{4}$$

$$n_{ll} = \frac{zn_l - n_{gl}}{2}, \tag{5}$$

$$n = n_g + n_l, \tag{6}$$

the coordination number $z = 6$. The chain–chain, gel–fluid interaction parameter is the difference between the Gibbs free energy associated with a gel–fluid contact and the average Gibbs free energy associated with a fluid–fluid and a gel–gel contact:

$$\omega = \epsilon_{gl} - (\epsilon_{gg} + \epsilon_{ll})/2. \tag{7}$$

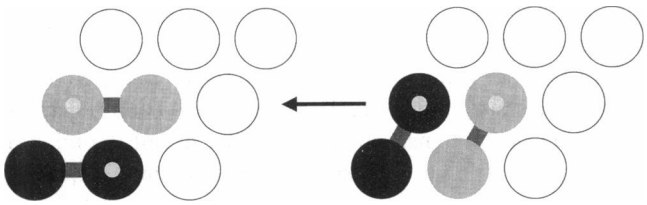

FIGURE 1 Illustration of the dimer move. Two lipids side by side (black and gray) participate in the move. Dots in two of the chains are meant only to allow following the movement of each chain.

Assuming that all ϵ_{ij} are enthalpic in origin, the enthalpy and entropy changes associated with the transition are

$$\Delta\mathcal{H} = (\mathcal{H}_l + 3\epsilon_{ll}) - (\mathcal{H}_g + 3\epsilon_{gg}), \quad (8)$$

$$\Delta\mathcal{S} = \mathcal{S}_l - \mathcal{S}_g. \quad (9)$$

Using Eqs. 1–8, we can write the Gibbs free energy of any configuration as

$$G = n(\mathcal{G}_g + 3\epsilon_{gg}) + n_l(\Delta\mathcal{H} - T\Delta\mathcal{S}) + n_{gl}\omega, \quad (10)$$

and the excess Gibbs energy change at any temperature is

$$\Delta G = n_l(\Delta\mathcal{H} - T\Delta\mathcal{S}) + n_{gl}\omega. \quad (11)$$

It thus follows that, in each step, the Gibbs free-energy change is

$$\delta G = \delta n_l(\Delta\mathcal{H} - T\Delta\mathcal{S}) + \delta n_{gl}\omega, \quad (12)$$

where δn_l and δn_{gl} are the changes in the number of fluid molecules and in the number of unlike nearest-neighbor contacts, respectively, in a change in the state of the system. $\delta n_l = 0, \pm 1$ for the dimer move and the Glauber step, respectively.

Finally, the heat capacity is given by the fluctuation-dissipation theorem (Hill, 1960)

$$C_p = \frac{\langle H^2\rangle - \langle H\rangle^2}{kT^2} \quad (13)$$

$$= \frac{(\Delta\mathcal{H})^2\langle(\Delta n_l)^2\rangle + \omega^2\langle(\Delta n_{gl})^2\rangle}{kT^2}, \quad (14)$$

where $\langle(\Delta n_l)^2\rangle$ and $\langle(\Delta n_{gl})^2\rangle$ are the variances in the number of fluid chains and gel–fluid contacts at each temperature.

The Monte Carlo calculations require the input of a few parameters: the gel–fluid enthalpy change ($\Delta\mathcal{H}$), the main transition temperature (T_m), and the chain–chain, gel–fluid interaction free energy (ω). The entropy change is calculated from $\Delta\mathcal{S} = \Delta\mathcal{H}/T_m$. We used for the enthalpy change/mol-lipid (8.7 kcal/mol-lipid) and the temperature (310.3 K) of the gel–fluid phase transition the corresponding values experimentally obtained with small unilamellar vesicles (SUVs) of dipalmitoylphosphatidylcholine (DPPC). The only free parameter in these calculations is the chain–chain (or lipid–lipid) interaction free energy, which is adjusted so that the maximum in the calculated excess heat capacity function would match that of the experimental heat-capacity function for DPPC SUVs.

RESULTS

Three different types of Monte Carlo (MC) simulation were performed: i) assuming that the acyl chains are independent both chemically and thermodynamically and only Glauber steps are used; ii) using coupled dimers, in which case both chains of each dimer are chemically and thermodynamically coupled and both Glauber steps and dimer move steps are used; and iii) using uncoupled dimers, in which case the chains of each dimer are chemically linked but thermodynamically uncoupled (here both types of step are also needed).

The transition probabilities were calculated as described. Equilibrium, as judged by constancy in the average value of the enthalpy, was generally achieved in fewer than 2000 MC cycles. However, simulations were carried out over 32,000 MC cycles to yield good statistics. Attainment of equilibrium was also demonstrated by the facts that identical distributions were obtained regardless of the initial configuration of the lattice (e.g., totally gel, totally fluid, or a random mixture) and that the value of the enthalpy calculated from the integral of the heat-capacity curve was in agreement with that obtained from the average value obtained from the MC simulation at a given temperature.

The excess heat capacity as a function of temperature calculated by use of independent or coupled chains (Fig. 2, open circles) is in good agreement with the experimental one for DPPC SUVs. The experimental heat-capacity function is a rather severe test of a model to represent a system accurately because both the shape of the function and its integral are well defined. We had originally (Suurkuusk et al., 1976) estimated that the enthalpy change for the gel–fluid phase transition of DPPC SUVs was 6.1 kcal/mol. This estimate was based on early data that used a mixed population of SUVs and large unilamellar vesicles, the result of SUV fusion in the gel state. The more recent data shown in Fig. 2 were obtained with a preparation containing no more than 5% large unilamellar vesicles. Sugar et al. (1994) were unable to reproduce even the normalized shape of the curve

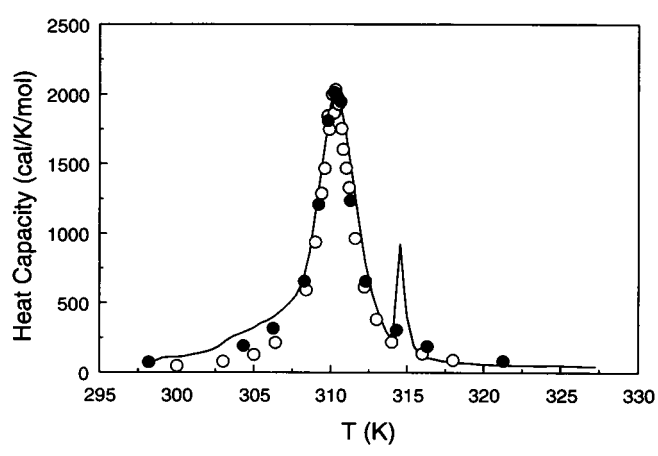

FIGURE 2 Comparison of the simulation results with the experimental excess heat capacity of DPPC SUVs. The solid curve is the experimental curve, open circles are results for dimers (thermodynamically coupled chains), and filled circles are the results for lattice points representing whole lipids from Sugar et al. (1994). The sharp peak at 315 K is due to the melting of multilamellar vesicles, which are formed by fusion of the SUVs (Suurkuusk et al., 1976). In this particular sample they represent less that 5% of the total lipid.

if the enthalpy change was assumed to be 6.1 kcal/mol but could accurately reproduce the heat capacity function if the accepted value for large unilamellar vesicles of 8.7 kcal/mol was used and if the lattice sites were assumed to represent an entire phospholipid molecule (Fig. 2, filled circles).

To match the maximum of the experimental excess heat capacity, which occurs at $T = 310.3$ K in DPPC SUVs (Suurkuusk et al. 1976), the unlike nearest-neighbor interaction free energy ω was adjusted as indicated in Table 1. This interaction parameter can be considered as composed of an enthalpic and an entropic contribution,

$$\omega = \omega_H - T\omega_S, \tag{15}$$

but if the transition region is narrow, as it is in the present case, the results are identical whether they are taken as being exclusively entropic or exclusively enthalpic. We indicate both values in the table. The value used for the enthalpy of the transition was always that experimentally obtained, $\Delta H = 8.7$ kcal/mol-lipid (Cevc, 1993) or 4.35 kcal/mol-chain.

We have tested different ratios of Glauber and dimer move cycles. In the range 1:1–1:10 of the number of cycles of dimer versus Glauber steps no significant differences were observed in any of the calculated properties. The system consisting of 60×60 chains typically equilibrated in 2000 MC cycles, but data collection for subsequent statistical analysis was not initiated until 5000 MC cycles were carried out. Inclusion of the new step did not affect the equilibration time appreciably. We have performed simulations on systems in which the number of dimer lipids was 450 (30×30 lattice sites), 1800 (60×60 lattice sites), and 7200 (120×120 lattice sites). We have also performed simulations, at five different temperatures, in which the two types of move were random rather than sequential. The major difference in the results was that the heat capacity appears to be greater by 2–4%, well within the error of the calorimetrically measured ΔH used in the calculation.

With the choice of parameters indicated above, the behavior of the average properties was essentially identical for independent and connected chains. In addition, the interaction parameter and the specific heat function were independent of system size, which means that the transition is not a first-order phase transition. This is also evident from Fig. 3, where the distribution functions at three different temperatures are shown. These distribution functions exhibit single maxima rather than two, which would be expected for a first-order phase transition. The same conclusion regarding the nature of the transition is reached by use of the relation between the critical temperature and the unlike nearest-neighbor interaction parameter in an Ising model. For a triangular lattice and in our notation, that relation is $kT_c/\omega = 0.912$ (Fisher, 1967; Doniach, 1978), where T_c is the critical temperature. Using our value $\omega = 300$ cal/mol for independent chains gives $T_c = 137$ K. As $T_m > T_c$ ($T_m = 310.3$ K) the transition is not first order. Recently Corvera et al. (1993) showed that the transition was not first order when they used the parameters of a 10-state Pink model. It was subsequently shown that first-order behavior could be obtained if a mismatch interaction between lipids in different conformational states were introduced into the Hamiltonian (Zhang et al., 1992). This is analogous to an increase in the value of our interaction parameter (Heimburg and Biltonen, 1996).

TABLE 1 Thermodynamic parameters used in the simulations

Lattice Sites	ω_H ($\omega_S = 0$)	ω_S ($\omega_H = 0$)
Whole lipids*	282.4 cal/mol-lipid	−0.91 cal/K/mol-lipid
Independent chains#	300 cal/mol-chain	−0.97 cal/K/mol-chain
Coupled chains (dimers)	174.6 cal/mol-chain	−0.56 cal/K/mol-chain

*In Sugar et al. (1994) the values for the interaction parameters are said to refer to mol-chain, which is a typing mistake; those values refer to mol-lipid.

#The same result is obtained for chemically coupled (covalently linked) or chemically uncoupled chains, as long as they are thermodynamically uncoupled.

Other statistical properties of the systems such as the mean cluster size, the cluster size distribution, and the fraction of molecules or chains in the largest fluid (or gel) cluster have been calculated by the procedure outlined by Sugar et al. (1994). In all cases, essentially identical results were obtained, regardless of the lattice representation used. This is demonstrated in Fig. 4 for the mean fluid cluster size as a function of temperature, which diverges as the percolation threshold is approached. In the calculation of the mean cluster size, percolated clusters were included. Although the number of small clusters is most frequent, the majority of the chains are to be found in the largest clusters, as can be seen in the snapshots shown in Fig. 5.

The percolation probabilities for coupled and independent chains as a function of fluid fraction are indistinguishable (Fig. 6). The percolation threshold of the fluid phase is estimated to be 0.52 for both dimers and monomers. Moreover, the steepness of the percolation transition is the same. Note for reference that (without interactions) the site percolation threshold is exactly 0.5 for points (Stauffer and Aharony, 1992) and 0.486 for dimers (Saxton, 1993) in a triangular lattice.

The number of gel–fluid contacts was different in the simulations that used monomers or infinitely coupled dimers. However, this is simply because each chain in a coupled dimer always has one like nearest neighbor (its partner in the dimer).

DISCUSSION

If a lattice is occupied by dimers instead of monomers an additional restriction is imposed on how a Monte Carlo simulation should be performed to explore the entire conformational space. One of the possible ways would be to introduce vacancies, multiple occupancy of lattice sites, or both, any of which would allow for movement of dimers.

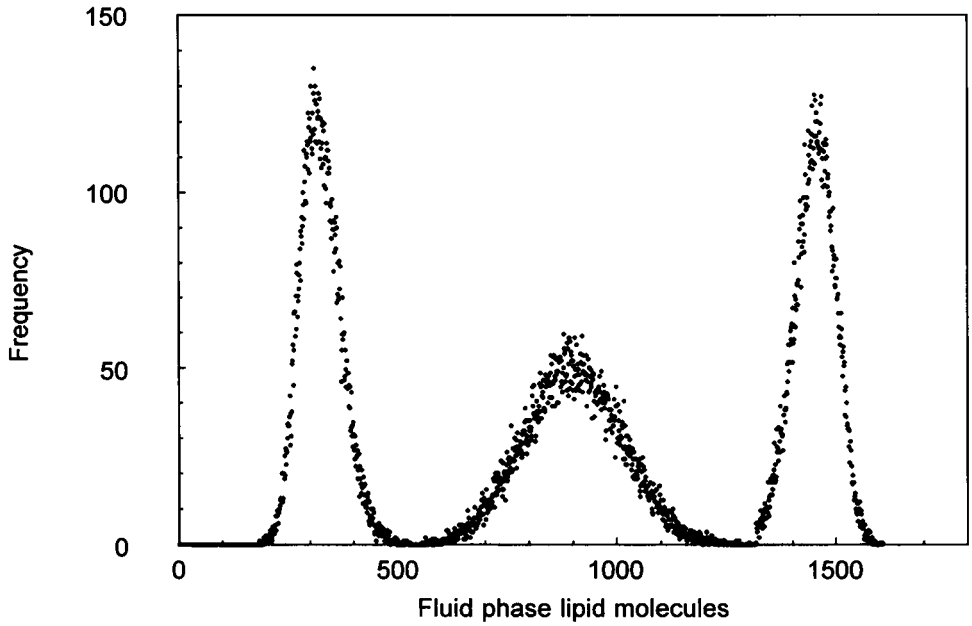

FIGURE 3 Distribution function for fluid lipid molecules for dimers (thermodynamically coupled chains) at three different temperatures: left, 308.3 K; center, 310.3 K (T_m); right, 312.3 K. Similar behavior is observed with the other models.

However, using lattice vacancies would require the introduction of new parameters in the simulation. An alternative approach to the goal of moving the dimers throughout the lattice, which we have chosen, is to keep the lattice fully occupied and use simultaneous movement of several dimers. The standard Kawasaki step (Binder, 1979) applied to exchange of two dimers is not sufficient because the initial orientation of dimers on the lattice remains "frozen."

The dimer move step that we introduce involves two dimers that define the four corners of a rhombohedron. The move changes the orientation of dimers within this rhombohedron. There are several ways in which this could be done, but in our simulation we considered only the move that seems physically the most plausible. It is interesting to note that there exists a conformation of dimers on the lattice for which no simultaneous move of two dimers is possible. This is the ordered conformation in the herringbone pattern that has been observed by x-ray diffraction in many phospholipid crystals (Pascher et al., 1981; Hauser et al., 1981). Our system is thus, strictly speaking, nonergodic, because such an ordered conformation cannot be reached. We have never encountered any significant part of the lattice in such a conformation, which must be entropically unfavorable.

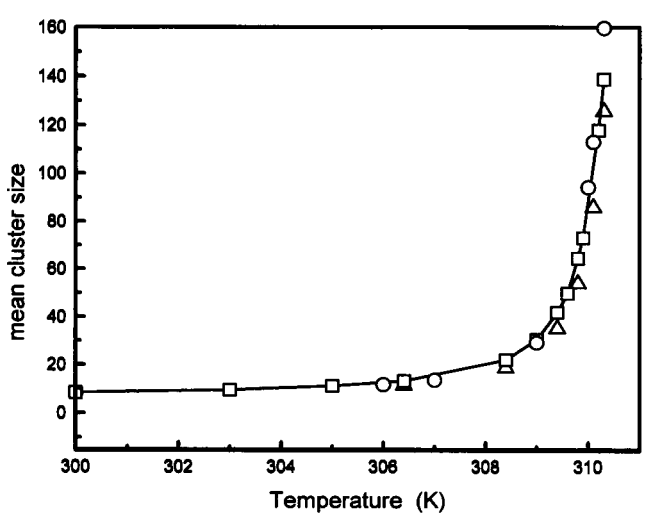

FIGURE 4 Mean fluid phase cluster size as a function of temperature for dimers with thermodynamically coupled chains (○), dimers with independent chains (△), and whole lipids (□). The cluster size is expressed in terms of number of lattice sites in all cases.

Inasmuch as we were interested primarily in equilibrium properties and not in diffusion, we could have chosen any other type of move that would enable the system to reach any possible conformation. However, the dimer move that we introduced seems plausible for a diffusion model within the approximation that phospholipid chains can be treated as cylinders (or close-packed disks in two dimensions). The move described is that which, besides preserving dimer covalent links, leads to least overlap with neighboring chains during the move. It would be interesting to compare diffusion calculations by using this model with molecular dynamics simulations. However, simulations of this type involving phospholipids published recently (Venable et al., 1993) were done on a time scale that is significantly shorter than that of diffusion.

The present study indicates that, in Monte Carlo calculations of the gel–fluid phase transition of one-component lipid systems, there is essentially no difference in treating phospholipids as independent chains, as monomers, or as dimers in a lattice. This result is important because it removes one type of criticism of the validity of Monte Carlo simulations of lipid membranes that were performed in the past that considered the acyl chains independent or considered that the lipid molecules occupy the sites of a lattice of coordination number 6, both possibilities being strictly in-

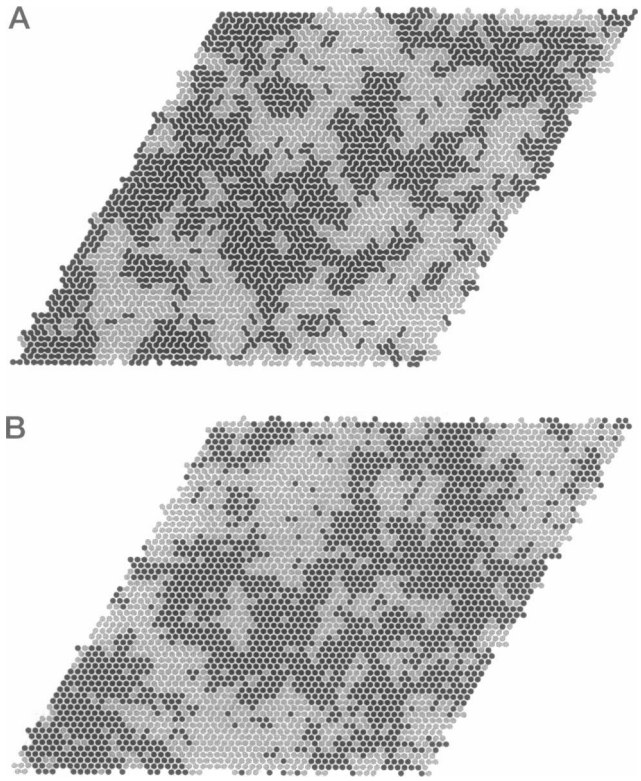

FIGURE 5 Snapshots of the simulations (*A*) with dimers with thermodynamically coupled chains and (*B*) with dimers with independent chains, at $T = 310.3$ K. Black dots represent fluid chains, and gray dots represent gel chains. The fraction of fluid-phase chains is 0.501 in (*A*) and 0.512 in (*B*). The number of gel–fluid chain contacts is 2348 in (*A*) and 2800 in (*B*).

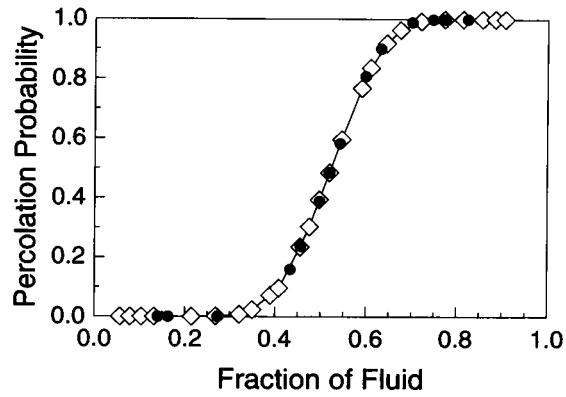

FIGURE 6 Percolation probability as a function of the fraction of fluid phase chains for dimers with thermodynamically coupled chains (*open symbols*) and for dimers with independent chains (*filled symbols*). The percolation threshold is 0.52 in both cases. The curve is to guide the eye.

correct. Because a significant effort has been devoted to those calculations it is important to verify that the results are essentially not affected by the aforementioned problems. The small differences in the results obtained in the two kinds of simulation are due mainly to the absence of the smallest clusters in the case of coupled acyl chains (Fig. 5). The exact values of the interaction parameters deduced from Monte Carlo calculations are model dependent, but they are all of the order of 300 cal/deg-mol-lipid, which is approximately half of the thermal energy at biologically relevant temperatures.

It has been shown by molecular dynamics simulations of lipid bilayers that the isomerization rates of both chains of the phospholipid molecules are coupled to a large extent (Venable et al., 1993). In general, the experimental heat capacity results of lipid bilayers indicate the existence of strong chain–chain interactions. Moreover, the differential scanning calorimetric results of Huang and co-workers (Lin et al., 1991) demonstrate that the transition retains a high degree of cooperativity even as the mismatch between the acyl chains is increased. It is thus likely that chains attached to the same headgroup are more strongly correlated thermodynamically and structurally than chains on different molecules. Therefore, treating the chains as coupled (both chemically and thermodynamically) is probably the best choice, and the value obtained in this case for the unlike nearest-neighbor interaction parameter is probably the best estimate within the limitations of the Ising model. The ratio of the interaction parameter for independent versus coupled chains (175 versus 300 cal/mol-chain) is approximately proportional to the ratio of the number of neighbor contacts of a single chain (6) to that of a dimer (10).

We have investigated here single-component membranes only. It is possible that for single-component systems in which the two acyl chains are not identical and for multicomponent systems the consideration of coupled instead of independent chains could have larger effects, particularly in the formation of small clusters above the transition region (Jorgensen and Mouritsen, 1995). We intend to examine such systems in the near future.

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