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Estimation of the Lateral Distribution of Molecules in Two-Component Lipid Bilayers†

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ABSTRACT: A new formalism to investigate the lateral distribution of molecules in lipid bilayers has been developed, and the results have been applied to the case of phosphatidylcholine mixtures. It is demonstrated that the experimental phase diagrams for these mixtures can provide the necessary information to generate computer-simulated bilayers with the desired molecular interactions and lattice constraints. Analysis of these computer-generated lipid bilayers allows calculation of the number of contacts between like and unlike molecules, the average size and number of compositional clusters, and the pair correlation functions. The results of this analysis provide a full quantitative description of the molecular organization of phosphatidylcholine within the plane of the bilayer.

Many physical and functional properties of biological membranes depend on the particular way in which the various components of the membrane are organized and distributed within the bilayer (Thompson & Huang, 1978). Furthermore, changes in the equilibrium distribution of membrane components have been associated with a variety of biological phenomena such as cell fusion (Papahadjopoulos et al., 1976), clustering of receptor sites (Taylor et al., 1971), and "capping" and "patch" formation (Raff & De Petris, 1973; Fishman & Brady, 1976). Studies on model membrane systems have demonstrated that the distribution of lipid components along the plane of the membrane is not physicochemically ideal and as such it is characterized by the presence of compositionally different domains (Shimshick & McConnell, 1973; Van Dijck et al., 1978; Correa-Freire et al., 1979). It has also been demonstrated that this distribution can be altered by changes in external variables and by changes in the physical state of the lipid molecules (Wallace & Engelman, 1978). Unfortunately, since a quantitative characterization of the lateral distribution of components in lipid bilayers has been unavailable, it has been impossible to establish quantitative correlations between the dynamic organization and functional properties of biological membranes.

In this communication the results of a computer analysis of the lateral distribution of molecules in phosphatidylcholine mixtures are presented. These semiempirical calculations are based upon the nonideality parameters obtained experimentally from the phase diagrams of these mixtures. These parameters are used to simulate bilayer lattices with a computer, by using a newly developed method. Direct analysis of the comput-

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er-generated lattices yields the lateral distribution of molecules as a function of the composition, the bilayer size, and the magnitude of the interaction energies.

Theory

The lateral distribution of molecules in a lipid bilayer containing N molecules of which N_A are of type A and N_B are of type B is dictated by the energetics of the molecular interactions between A and B molecules. In a two-component lipid bilayer there are three different types of contacts or interactions between molecules: A–A, B–B, and A–B. In all, there are $ZN/2$ contacts in the entire system (Rice, 1967):

$$\frac{ZN}{2} = N_{AA} + N_{BB} + N_{AB} \tag{1}$$

where Z is the coordination number of the lattice. For convenience we shall consider a simplified model where each molecule has precisely $Z = 6$ nearest neighbors which for lipid molecules with two hydrocarbon chains is the average of the minimum number, $Z_{min} = 4$, and the maximum number, $Z_{max} = 8$ (Lee, 1975; McCammon & Deutch, 1975). The total number of A–A and B–B contacts is equal to

$$N_{AA} = \frac{Z}{2}N_A - \frac{1}{2}N_{AB} \tag{2a}$$

$$N_{BB} = \frac{Z}{2}N_B - \frac{1}{2}N_{AB} \tag{2b}$$

The energy of the system is determined by the energy and number of A–A, B–B, and A–B contacts and can be written in the form

$$E = \frac{Z}{2}N_A E_{AA} + \frac{Z}{2}N_B E_{BB} + N_{AB}\Delta E_m \tag{3}$$

where

$$\Delta E_m = E_{AB} - \frac{1}{2}E_{AA} - \frac{1}{2}E_{BB} \tag{4}$$

ΔE_m, the nonideal energy term, can be identified as the energy required for the formation of one A–B bond out of A–A and B–B bonds and as such determines the degree of mixing of the two components. If ΔE_m is positive, the formation of A–B bonds will be difficult and like molecules will have the tendency to group together, forming relatively large clusters. If, on the other hand, ΔE_m is negative, A–B bonds will be energetically preferred over A–A and B–B bonds and the size of the clusters will approach a minimum. The case in which $\Delta E_m = 0$ defines the ideal mixture. In this case all the configurations of the system are energetically equivalent and the lipid molecules will be distributed randomly within the bilayer.

The configurational partition function of the system described above can be written as

$$Q_c = q_A^{N_A} q_B^{N_B} \sum_{N_{AB}} g(N,N_A,N_{AB}) e^{-N_{AB}\Delta E_m/RT} \tag{5}$$

where

$$q_A \equiv \exp\left(-\frac{Z}{2}\frac{E_{AA}}{RT}\right); \quad q_B \equiv \exp\left(-\frac{Z}{2}\frac{E_{BB}}{RT}\right) \tag{6}$$

$g(N,N_A,N_{AB})$ is equal to the number of ways of distributing N_A molecules of type A and N_B molecules of type B with a fixed number of A–B contacts. It is this combinatorial term which is difficult to evaluate mathematically, thus preventing an exact determination of the partition function. In general, the partition function Q_c can only be evaluated exactly for the ideal solution, in which case $g = N!/N_A!N_B!$. For finite ΔE_m, some approximate method is necessary in order to evaluate Q_c. The simplest one assumes that the molecules are randomly distributed even though ΔE_m is finite. This assumption constitutes the basis for the theory of regular solutions (Münster, 1974) and has been applied to the study of lipid mixtures by several authors [see Lee (1977) for an extensive review]. This theory, however, is not very useful if one is primarily concerned with the study of nonrandom distributions of molecules. More accurate treatments involve the introduction of approximate expressions for $g(N,N_A,N_{AB})$. Recently, Von Dreele (1978) has used the Prigogine approximation for the combinatorial term $g(N,N_A,N_{AB})$ in order to describe the degree of nonideality in the gel and liquid-crystalline phases of phosphatidylcholine mixtures. This approximation provides good estimates for the number of A–A, B–B, and A–B contacts and, as such, can be used to select the theoretical lipid distributions which predict a macroscopic behavior consistent with that observed experimentally.

A different approach to the mixing problem involves the use of molecular lattices generated with the help of a computer. These computer methods overcome the problem of evaluating $g(N,N_A,N_{AB})$ and have the advantage that the calculation of all quantities of interest reduces to a simple counting problem. These methods have proven to be extremely helpful to study critical phenomena in two-dimensional and three-dimensional systems in which exact mathematical solutions are unavailable (Binder, 1975, 1979; Sur et al., 1976; Stoll & Domb, 1978). In this communication an algorithm which allows very fast computer simulation of nonrandom distribution of molecules in very large lattices ($N > 10^4$) will be presented. This method is particularly suitable to study the lateral distribution of molecules in lipid bilayers in terms of the composition, the bilayer size, and the magnitude of the interaction energies between components.

Computer Generation of Lipid Bilayers. A lipid monolayer of $m \times n = N$ lipid molecules can be represented by a $m \times n$ matrix in which each matrix element $D_{i,j}$ represents a lattice position which can be occupied by a lipid molecule of type A or a lipid molecule of type B. The hexagonal packing of the lipid molecules is specified by proper assignment of the nearest-neighbor positions. Each matrix element $D_{i,j}$ can assume one of eight different values, depending on whether or not the site is occupied by an A or B molecule and the number of like molecules occupying nearest-neighbor positions. The absence of edges in a lipid vesicle is mimicked by imposing periodic boundary conditions on the lattice.

A typical computer experiment is initialized with all the lattice positions occupied by lipid molecules of the same type (A molecules). Then a second lipid molecule, B, progressively replaces A molecules until the bilayer contains only B molecules. The exact position of each substitution is obtained with a random number generator subroutine. Neighbor–neighbor interactions are introduced by increasing or decreasing the relative intrinsic probability of a substitution in one of the neighboring positions of a site already occupied by a B molecule. This excess probability is called P and is related to the nonideal energy ΔE_m by the expression $P = \exp[\Delta E_m/(RT)]$. With these definitions, each $D_{i,j}$ may assume one of the following values: (1) $D_{i,j} = 0$, if the site is occupied by a B molecule; (2) $D_{i,j} = P^k$, if the site is occupied by an A molecule and k nearest neighbors are B molecules. In this way, molecular distributions as a function of composition and neighbor–neighbor interactions can be obtained. The resulting computer output is a $m \times n$ matrix in which the sites occupied by B molecules are equal to zero. This matrix is stored and

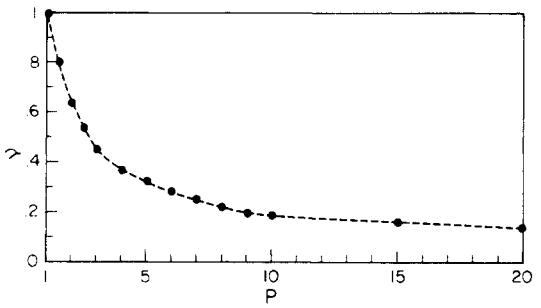

FIGURE 1: Calculated dependence of the nonideality parameter, ν, at $X_A = X_B = 0.5$ on the intrinsic excess probability for establishing a contact between two like molecules.

then analyzed by using a modified version of the method described by Müller-Krumbhaar (1979). This counting algorithm creates a second matrix in which all B molecules belonging to the same cluster are labeled with the same number. In this way, each compositional cluster within the lattice is identified by a unique cluster number. The resulting matrix of cluster numbers is then analyzed row by row, to yield the number of A–A, A–B, and B–B contacts, the cluster distribution, and the particle pair correlation function. The accuracy of this method has been tested by comparing the results of the simulations with exact values obtained for an infinite Ising lattice and with Monte Carlo calculations existing in the literature. For an infinite triangular lattice the exact critical concentration is $X_c = 0.5$ (Sykes & Essam, 1963); for a 100 × 100 lattice our calculations yield $X_c = 0.495$ which compares favorably with the value $X_c = 0.493$ obtained with Monte Carlo calculations (Essam, 1972). Recently, Hoshen & Kopelman (1976) have estimated $X_c = 0.4995$ for a 2000 × 2000 lattice.

It must be noted that the output of each computer experiment represents an instantaneous picture of a single lipid bilayer of the specified size and molar composition. This bilayer is not the "average" bilayer and as such the macroscopic properties of the system are calculated by averaging the results of several computer experiments. In this sense, this method performs an ensemble averaging of the properties of the system as opposed to the "time" averaging performed by Monte Carlo calculations (Binder, 1979). The number of computer experiments required for obtaining the macroscopic average of a thermodynamic function is determined by the condition of a vanishing variation of the calculated average with respect to the number of computer runs. In this way, ensemble fluctuations in the thermodynamic properties of the system can also be calculated.

Results

The phase diagrams of phosphatidylcholine mixtures have been extensively studied, both from an experimental (Shimshick & McConnell, 1973; Mabrey & Sturtevant, 1976) and a theoretical (Jacobs et al., 1977) point of view, and as such they constitute an appropriate system in which to test the present approach. Recently, Von Dreele (1978) has analyzed these phase diagrams in terms of the Prigogine approximation to the partition function of mixing and estimated the number of A–A, B–B, and A–B contacts in the gel and liquid-crystalline phases of DMPC–DPPC,[1] DMPC–DSPC, and DPPC–DSPC mixtures. This author expressed the deviations from ideality in terms of the nonideality parameter ν, defined as

$$\nu = \frac{N_{AB}(\text{real})}{N_{AB}(\text{ideal})} \tag{7}$$

This nonideality parameter, ν, can be calculated from the experimental phase diagram and is, by definition, equal to 1 for ideal mixing and less than 1 for $\Delta E_m > 0$. Within the context of this theory, ν is a function of P even though no exact theoretical relation exists. Nevertheless, an empirical relation can be obtained by explicitly counting the number of A–B contacts in computer-simulated lattices generated with different P values. The results of such calculations are presented in Figure 1. In these and all subsequent calculations the reported values are the mean of at least 10 different computer experiments. This number was chosen because further increase in the number of computer runs did not produce significant changes (<1%) in the statistical averages.

Table I: Nonideality Parameters for Phosphatidylcholine Mixtures

lipids	gel phase		liquid-crystalline phase	
	ν^a	P^b	ν^a	P^b
DMPC-DPPC	0.76	1.65	0.9	1.25
DMPC-DSPC	0.46	3.0	0.86	1.35
DPPC-DSPC	0.68	1.87	0.80	1.51

[a] Nonideality parameter ν at a 1:1 mole ratio; from Von Dreele (1978). [b] Excess probability for establishing a contact between like molecules; see text for details.

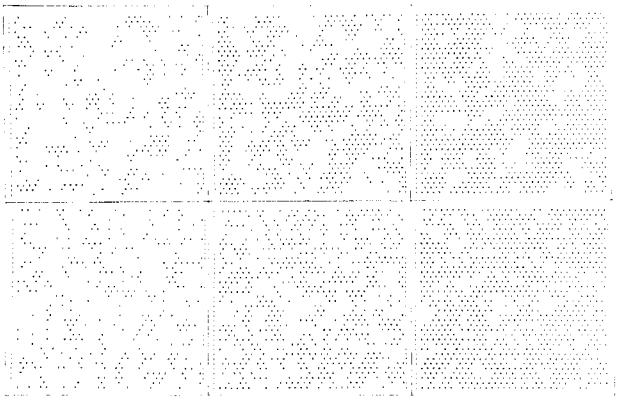

FIGURE 2: Typical computer simulation of the lateral distribution of molecules for DMPC–DPPC in the gel (top row) and liquid-crystalline phases (bottom row) at 0.25, 0.5, and 0.75 mole fraction of DMPC.

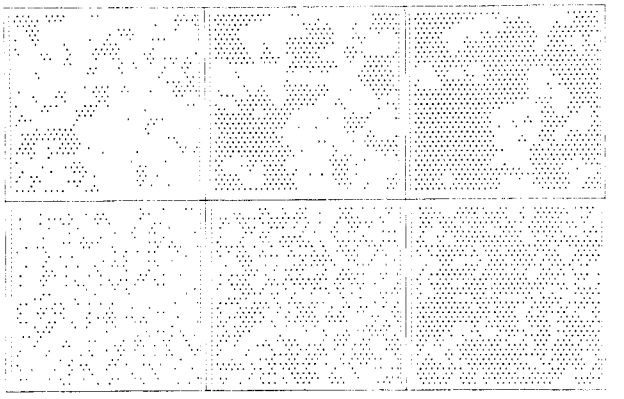

FIGURE 3: Typical computer simulation of the lateral distribution of molecules for DMPC–DSPC in the gel (top row) and liquid-crystalline phases (bottom row) at 0.25, 0.5, and 0.75 mole fraction of DMPC.

[1] Abbreviations used: DMPC, 1,2-dimyristoyl-3-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DSPC, 1,2-distearoyl-3-*sn*-phosphatidylcholine.

Summarized in Table I are the calculated P values for the gel and liquid-crystalline phases of DMPC–DPPC, DMPC–DSPC, and DPPC–DSPC. These P values were obtained from Figure 1 by using the ν values calculated by Von Dreele (1978) and were used to generate the corresponding lipid distributions. In Figures 2 and 3, typical computer simulations for DMPC–DPPC and DMPC–DSPC in the gel and liquid-crystalline phases are presented. As can be observed in Figures 2 and 3, the shape of the compositional clusters deviates largely from a compact or circular geometry. This is a consequence of the low P values characterizing these mixtures. In these cases, the approximate formulas (Fisher, 1967; Kanehisa & Tsong, 1978; Freire & Biltonen, 1978) relating the perimeter to the size of the clusters on the assumption of a compact or circular geometry are not strictly correct. For large P values these formulas accurately represent this relation (Stoll et al., 1972). As is apparent from the figures, the deviation of the DMPC–DPPC mixtures from the ideal behavior is small for both the gel and liquid-crystalline phases. DMPC and DSPC mixtures, on the other hand, are highly nonideal in the gel phase, giving rise to the formation of well-defined compositional domains. In the liquid-crystalline phase, however, DMPC–DSPC mixtures are very similar to DMPC–DPPC mixtures. Thus, the phase transition of DMPC–DSPC mixtures must be accompanied by large changes in the lateral distribution of these lipid molecules. These results qualitatively agree with previous interpretations of the experimental phase diagrams (Shimshick & McConnell, 1973; Mabrey & Sturtevant, 1976).

A quantitative characterization of these computer-generated lipid bilayers was obtained by calculating, in each case, the number of A–A, B–B, and A–B contacts, the pair correlation functions, and the mean number and size of A and B clusters. These computer simulations were made by using matrices of 34 × 38, 50 × 50, and 100 × 100 lipid molecules; however, no size effects were observed in this size range. This was a consequence of the absence of molecular correlations of the order of the bilayer size due to the small values of P for these mixtures and the absence of edge effects due to the periodicity of the lipid bilayers.

Relative Number of A–A, B–B, and A–B Contacts. The number of contacts between like and unlike molecules is an important measure of the degree of mixing of A and B lipid molecules. A convenient way of expressing these quantities is in terms of the normalized pairs F_{AA}, F_{AB} and F_{BB}, F_{BA}. Each of these quantities is defined as the ratio between the actual number of contacts of a given type and the maximum possible number of contacts of that type and are given by

$$F_{AA} = \frac{2N_{AA}}{ZN_A} \qquad F_{AB} = \frac{N_{AB}}{ZN_A} \tag{8a}$$

$$F_{BB} = \frac{2N_{BB}}{ZN_B} \qquad F_{BA} = \frac{N_{AB}}{ZN_B} \tag{8b}$$

From eq 2 it follows that $F_{AA} + F_{AB} = 1$ and $F_{BB} + F_{BA} = 1$. The advantage of this representation is that, for the ideal mixing, all of these fractions are linear functions of the molar composition of the mixture, thus providing us with a simple way of estimating deviations from ideality over the entire composition range. The calculated fractions for DMPC–DPPC and DMPC–DSPC have been plotted in Figures 4 and 5 as a function of the mole fraction of DMPC.

Previously, Lee (1978) has estimated the quantity $X_{AA}/X_{AB} \equiv [F_{AA}/(1 - F_{AA})]$ at $X_A = 0.5$ for DMPC–DPPC, using the theory of athermal solutions. This author calculated $X_{AA}/X_{AB} = 1.69$ and $X_{AA}/X_{AB} = 2.89$ for the liquid-crystalline and gel phases of DMPC–DPPC, respectively; transformation of these

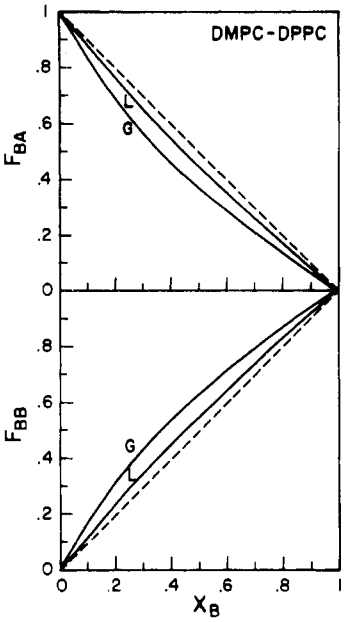

FIGURE 4: Relative amount of DMPC–DMPC (B–B) and DMPC–DPPC (B–A) contacts in the gel (G) and liquid-crystalline (L) phases as a function of the mole fraction of DMPC. The broken line is the ideal curve.

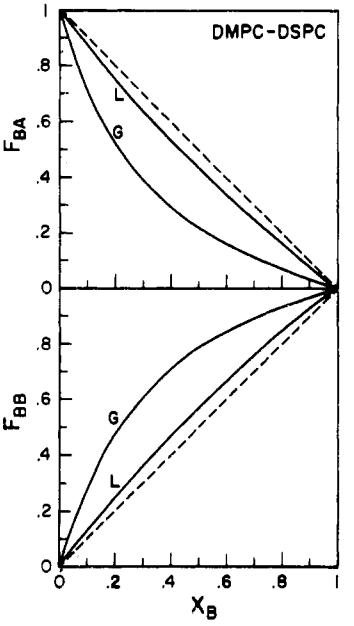

FIGURE 5: Relative amount of DMPC–DMPC (B–B) and DMPC–DSPC (B–A) contacts in the gel (G) and liquid-crystalline (L) phases as a function of the mole fraction of DMPC. The broken line is the ideal curve.

quantities yield $F_{AA} = 0.62$ and $F_{AA} = 0.74$ which should be compared with our values of $F_{AA} = 0.55$ and $F_{AA} = 0.62$ for the same system. As expected, the F_{AA} values calculated here lie between those calculated with the theory of athermal solutions and the value $F_{AA} = 0.5$ calculated with the theory of regular solutions for both phases. The theory of athermal solutions overestimates the number of A–A and B–B contacts due to the assumption that all deviations from ideality arise from a nonrandom distribution of molecules. Conversely, the theory of regular solutions underestimates N_{AA} and N_{BB} due to the assumption of random mixing.

Pair Correlation Function. The ordering of the lipid molecules within the plane of the bilayer can be expressed in terms of the pair correlation function, $g_{i,i}(d)$, relating the

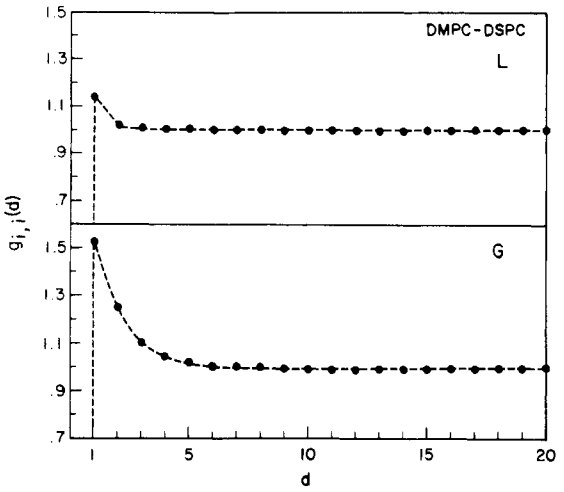

FIGURE 6: Pair correlation function $g_{i,i}(d)$ as a function of the separation distance, d (in number of molecules), for DMPC–DSPC in the gel (G) and liquid-crystalline (L) phases. It was calculated at $X_{DMPC} = X_{DSPC} = 0.5$.

probability $P_{i,i}(d)$ that two lattice positions separated by a distance d are occupied by molecules of the same type. For a random distribution of molecules, the occupancies of two lattice positions are uncorrelated and $P_{i,i}(d)$ is independent of the separation; in this case

$$P_{i,i}(d) = X_i^2 \tag{9}$$

where X_i is the mole fraction of component i. For $\Delta E_m > 0$, lipid molecules of the same type will have the tendency to aggregate, forming compositionally different domains along the plane of the bilayer. In this case, $P_{i,i}(d)$ is no longer independent of the separation and can be written in the form (Green & Hurst, 1964)

$$P_{i,i}(d) = X_i^2 g_{i,i}(d) \tag{10}$$

The above equation defines the pair correlation function. As is apparent from the equation, $g_{i,i}(d)$ is greater than 1 if the probability of finding two like molecules separated by a distance d is larger than that of the ideal case and is equal to 1 when the two probabilities are the same. Generally, $g_{i,i}(d)$ has a maximum at the nearest-neighbor separation distance and decays to unity as the separation between the lattice positions increases. The total distance over which this decay process occurs defines the correlation length for the system and measures the distance over which the behavior of two molecules is not independent of each other. Beyond this characteristic distance the occupancies of two lattice positions are uncorrelated.

The behavior of $g_{i,i}(d)$ as a function of d for an equimolar mixture of DMPC and DSPC in the gel and liquid-crystalline phases is shown in Figure 6. In each case, $g_{i,i}(d)$ was calculated as follows: first, by directly counting the total number of like molecules separated by a distance d in the lattice and, second, by normalizing this number with respect to the number existing in a random arrangement of lipid molecules (see eq 10). In both phases, $g_{i,i}(d)$ has a maximum at the nearest-neighbor separation distance; however, the amplitude of this maximum is greater in the gel phase. In the gel phase $g_{i,i}(d)$ reaches its limiting value of unity at about $d = 6$ molecular diameters, whereas in the liquid-crystalline phase $g_{i,i}(d)$ is already equal to 1 at $d = 3$. These results indicate that the lipid molecules are more highly correlated in the gel phase and, in this phase, the influence of a single molecule on the organization of the mixture extends over longer distances. This is an important property of the system when considering

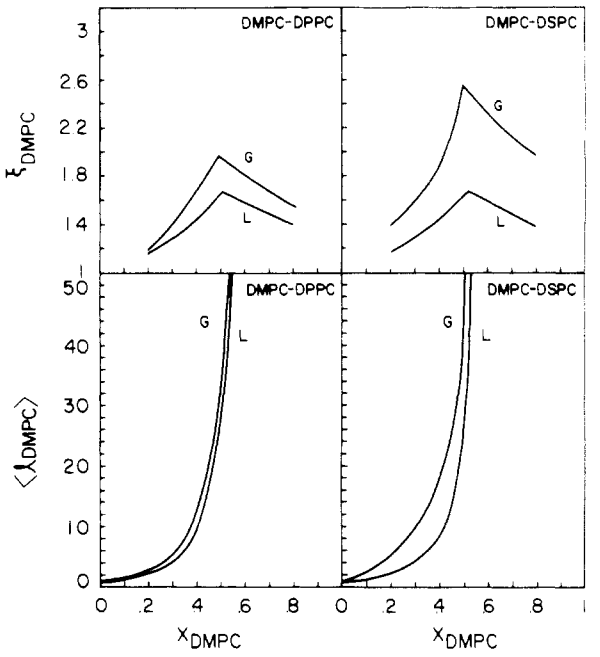

FIGURE 7: Correlation length, ξ_{DMPC}, and average cluster size, $\langle l_{DMPC} \rangle$, as a function of the mole fraction of DMPC for DMPC–DPPC and DMPC–DSPC mixtures in the gel (G) and liquid-crystalline (L) phases.

correlations between molecular fluctuations in different regions of the bilayer. The extent of these correlations is measured in terms of the correlation length, ξ_i, defined by (Münster, 1974)

$$\xi_i^2 = \frac{\sum_d d^2[g_{i,i}(d) - 1]}{\sum_d [g_{i,i}(d) - 1]} \tag{11}$$

In the top part of Figure 7 the calculated correlation lengths for DMPC in the gel and liquid-crystalline phases of DMPC–DPPC and DMPC–DSPC mixtures have been plotted as a function of the mole fraction of DMPC. In all cases the correlation length is maximal at $X_{DMPC} \simeq 0.5$, indicating that at this composition the magnitude of the local fluctuations is maximized.

Average Size of Compositional Clusters. The average size and number of A and B clusters are important quantities to consider since they provide a quantitative estimate of the presence of compositional domains within the plane of the bilayer. In a lipid monolayer of size N, the average size, $\langle l_i \rangle$, and the number of compositional clusters of type i are not independent of each other and are related by means of the equation (Freire & Biltonen, 1978)

$$\rho_i \equiv \frac{n_i}{N} = \frac{X_i}{\langle l_i \rangle} \tag{12}$$

where ρ_i is equal to the density of i clusters. These two quantities are strongly dependent on the molar composition of the mixture and the magnitude of the interaction energies between components. In the bottom part of Figure 7 the average size of DMPC clusters in the gel and liquid-crystalline phases of DMPC–DPPC and DMPC–DSPC mixtures have been plotted as a function of the mole fraction of DMPC. These quantities were calculated by directly counting the number of clusters in computer-generated bilayers.

As is apparent from Figure 7, the gel-to-liquid crystalline transition is accompanied by a decrease in cluster size and, therefore, by a parallel increase in the number of clusters. These changes are relatively small for DMPC–DPPC and

Table II: Average Cluster Size and Fraction of A-B Contacts in Equimolar Mixtures of Phosphatidylcholines

lipids	gel phase		liquid-crystalline phase	
	⟨l⟩	F_{AB}	⟨l⟩	F_{AB}
DMPC-DPPC	30	0.38	26	0.45
DMPC-DSPC	39	0.23	27	0.43
DPPC-DSPC	31	0.34	28	0.4

DPPC–DSPC mixtures (see Table II), but they are rather large for DMPC–DSPC. It should be noted, however, that the average cluster sizes do not necessarily provide by themselves an adequate physical representation of the bilayer. As can be deduced from Figures 2 and 3, the cluster size distributions of these mixtures are such that the average cluster sizes do not generally agree with the most probable sizes.

Discussion

The molecular organization of the lipid bilayer has been studied from a structural and functional point of view by several authors (Singer & Nicolson, 1972; Huang & Mason, 1978; Thompson, 1978). It is well-known, for example, that lipid and protein molecules are able to diffuse along the plane of the membrane and that some lipid components are able to move from one bilayer face to the other (McNamee & McConnell, 1973). This capability of the various membrane components to undergo different types of motion indicates that the biological membrane cannot be regarded as a rigid structure but rather as a dynamic entity whose molecular organization is capable of being altered by changes in the magnitude of the interaction energies between components. Since these interaction energies can be affected by external variables, it is important to develop a quantitative description of this distribution, in order to predict the configurational state of the bilayer under a variety of physicochemical conditions.

In these studies we have shown how the lateral distribution of phosphatidylcholines is governed by the interaction energies between components and how this distribution is affected by the physical state of the lipid bilayer. Within the context of the present approach, the molecular interaction energies are expressed in terms of the parameter P, defined as the excess intrinsic probability for establishing a contact between two like molecules. This excess probability is the only parameter required to generate molecular distributions and, as such, a one-to-one correspondence can in principle be established between the observed thermodynamic properties of the system and the molecular organization of the mixture.

The starting point of the present theory is the configurational partition function given by eq 5. This partition function is also the starting point for the theory of regular solutions, athermal solutions, and other theoretical treatments existing in the literature (Munster, 1974); in this sense, the present approach does not introduce new assumptions into the basic statistical mechanical description of binary systems.

As discussed in previous paragraphs, the various approximations to the mixing problem arise from the fact that the degeneracies of the energy states of the mixture cannot be enumerated exactly, therefore requiring some approximate expression for the combinatorial term $g(N,N_A,N_{AB})$. The approach developed in this communication overcomes this problem by practically reproducing the statistical ensemble with the computer. In this sense, the solution to the mixing problem is exact and the accuracy of the calculated averages depends only on the number of computer experiments used to estimate these quantities.

In these studies, the lateral distribution parameters for phosphatidylcholines were deduced from nonideality parameters calculated by Von Dreele (1978) from the experimental phase diagrams of these mixtures. However, the proposed method is not restricted to this type of data, and the required information can be obtained from any physical observable which is sensitive to the interactions between components and, therefore, capable of being used to estimate P. Previously, we have shown in a qualitative form (Correa-Freire et al., 1979; Freire & Biltonen, 1979) that the composition dependence of the enthalpy and entropy changes associated with the gel–liquid-crystalline transition of two-component lipid bilayers can be used to obtain information regarding the state of aggregation of lipid and membrane proteins. These studies prove that this information is readily available and that physicochemical data can be appropriately transformed to yield a complete description of the distribution of molecules in a two-component system.

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Identification of an Arginine Important for Enzymatic Activity within the Covalent Structure of Yeast Inorganic Pyrophosphatase†

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ABSTRACT: Previously we presented evidence for an essential arginine involved in binding inorganic pyrophosphate during catalysis by yeast inorganic pyrophosphatase [Cooperman, B. S., & Chiu, N. Y. (1973b) *Biochemistry 12*, 1676]. In the present work we show this residue to be arginine-77. Arginine-77 reacts with [^{14}C]phenylglyoxal considerably faster than the other five arginine residues in the enzyme subunit, and its reaction with phenylglyoxal is selectively blocked in the presence of the competitive inhibitor calcium pyrophosphate. Our procedure leading to the identification of Arg-77 utilizes the following steps: CNBr cleavage, digestion with *Staphylococcus aureus* V8 protease and with pepsin, and peptide mapping. All of these steps are performed below pH 5, a restriction imposed by the lability of the phenylglyoxal-arginine adduct at neutral pH. In related work, we find the model compound N^{α}-acetyl(diphenylglyoxal)arginine to hydrolyze 10 times more slowly at pH 4 than at pH 7. The high yields of derivatized peptides obtained in this work suggest the potential general utility of our procedure for locating arginine residues derivatized with phenylglyoxal within the covalent structure of proteins.

Despite their widespread distribution and central importance to cellular metabolism, phosphoryl-transfer enzymes remain incompletely understood with respect to their detailed mechanisms, especially when compared with what is known about more well studied enzymes, such as the serine proteases (Blow, 1976; Kraut, 1977). This situation is in the process of changing. In recent years, high-resolution X-ray structures have been reported for adenylate kinase (Pai et al., 1977), hexokinase (Anderson et al., 1978), and pyruvate kinase (Levine et al., 1978), and this new information, when coupled with the large body of information available from studies of these enzymes in solution, should lead to the formulation of more detailed models of enzyme mechanism. Yeast inorganic pyrophosphatase, EC 3.6.1.1 (PPase),[1] is another phosphoryl-transfer enzyme for which information is rapidly accumulating. The covalent structure has been determined (Cohen et al., 1978), two research groups have reported low-resolution crystal structures (Bunick et al., 1978; Makhaldiani et al., 1978), and a high-resolution structure determination is underway.[2] In addition, a fair amount is known about the stoichiometries and affinities of divalent metal ion, pyrophosphate and phosphate binding (Ridlington & Butler, 1972; Cooperman & Chiu, 1973a; Rapoport et al., 1973; Hamm & Cooperman, 1978; D. J. Hamm, B. Springs, and B. S. Cooperman, unpublished experiments), about the kinetic and chemical mechanisms (Rapoport et al., 1972; Moe & Butler, 1972; Konsowitz & Cooperman, 1976; Hackney & Boyer, 1978), about the orientation of metal ions and phosphate ligands at the active site (Hamm & Cooperman, 1978), and about the identity and roles of essential amino acid residues (Cooperman & Chiu, 1973b; Heitmann & Uhlig, 1974).

We and others (Cooperman & Chiu, 1973b; Heitmann & Uhlig, 1974) have previously shown that the arginine-specific reagent phenylglyoxal (PhGx) (Takahashi, 1968, 1977a,b) fully inactivates PPase in a pseudo-first-order process, that the rate constant for inactivation can be substantially decreased in the presence of competitive inhibitors, and that the modified PPase loses the ability to bind such competitive inhibitors. These results were taken as evidence for an essential arginine at the active site of PPase and are consistent with the known

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[1] Abbreviations used: BAWP, butanol-acetic acid-pyridine-water (15:3:10:12 v/v/v/v); NAcArg(PhGx)$_2$, N^{α}-acetyl(diphenylglyoxal)arginine; Arg(PhGx)$_2$, (diphenylglyoxal)arginine; PPase, yeast inorganic pyrophosphatase; PhGx, phenylglyoxal; SAV8P, *Staphylococcus aureus* V8 protease.

[2] D. Voet (personal communication).

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