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The effects of density fluctuations on the partitioning of foreign molecules into lipid bilayers: Application to anaesthetics and insecticides

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An extensive computer-simulation study is performed on a simple but general molecular model recently proposed (Jørgensen et al. (1991) Biochim. Biophys. Acta 1062, 227–238) to describe foreign molecules interacting with lipid bilayers. The model is a multi-state lattice model of the main bilayer transition in which the foreign molecules are assumed to intercalate at interstitial lattice positions. Specific as well as non-specific interactions between the foreign molecules and the lipid acyl chains are considered. Particular attention is paid to the fluctuating properties of the membrane and how the presence of the foreign molecules modulates these fluctuations in the transition region. By means of computer-simulation techniques, a detailed account is given of the macroscopic as well as microscopic consequences of the fluctuations. The macroscopic consequences of the fluctuations are seen in the thermal anomalies of the specific heat and the passive trans-membrane permeability. Microscopically, the fluctuations manifest themselves in lipid-domain formation in the transition region which implies an effective dynamic membrane heterogeneity. Within the model it is found that certain anaesthetics and insecticides which are characterised by specific interactions with the lipids have a strong effect on the heterogeneity of the membrane inducing regions of locally very high concentration of the foreign molecules. This leads to a broadening of the specific heat peak and a maximum in the membrane/water partition coefficient. These results are in accordance with available experimental data for volatile general anaesthetics like halothane, local anaesthetics like cocain derivatives, and insecticides like lindane.

1. Introduction

The partitioning of amphiphilic molecules into membranes is of importance for a range of biological processes. The ability of these compounds to perturb living systems has resulted in their widespread use in medicine and biology. Well known examples are anaesthetics, alcohols, halucinogens, and a range of drugs and pesticides. The underlying pharmacological mechanisms for the action of most of these compounds are still unknown.

Lipid bilayer membranes in the fluid phase are regarded as models for biological membranes. In consequence, their structural properties have been investigated in great detail both from the experimental and the theoretical point of view [1,2]. One of the more spectacular properties of lipid bilayers is the occurrence of a phase transition, known as the main transition, which has been observed in both multilamellar bilayers and large unilamellar vesicles composed of lipid molecules [1]. At the main transition, the lipid bilayer passes from a two-dimensional solid known as the gel phase, to a two-dimensional fluid, called the liquid-crystalline or fluid phase. The main transition implies major structural changes of the lipid bilayer

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonate; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine.

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which are of great importance for the functioning of membranes. In the present paper, we shall focus on this transition and study how foreign molecules affect the transition characteristics. We shall use such effects to gain insight into the nature of the interaction af foreign molecules with lipid membranes.

Experimental data show that the thermodynamic properties of lipid bilayers at the main transition, such as the change in bilayer area per molecule and the average acyl-chain order parameter, vary in a continuous though abrupt manner across the transition region (for a list of references, see Ref. 2). Furthermore, response functions, such as the the specific heat and the lateral compressibility, display apparent singularities at the transition. Hence, although probably of first order, the main transition is strongly dominated by thermal density fluctuations [2,3]. The presence of peaks in the specific heat and lateral compressibility as well as the anomaleous behavior of the passive transmembrane permeability are indeed macroscopic consequences of the strong fluctuations at the transition. Microscopically, the fluctuations at the main phase transition are associated with the formation of fluid domains in the gel phase below the main phase transition and gel domains in the fluid phase above the phase transition [4,5]. Domain formation is a dynamic process and the domains of correlated acyl chains fluctuate in size and position. This results in a fluctuation-induced lateral heterogenity which is charaterised by the interfacial region between the domains and the bulk phase. It has been postulated [4] that this interface forms a soft leaky region with poor packing characteristics as it is dominated by acyl-chain conformations with some degree of disorder. These properties of the interface lead to a stabilization of the domains which in turn allows the interfacial region to account for the physical properties of the bilayer in the transition region and give the transition itself a continous appearence [6], in agreement with experimental observations. Indeed the leaky nature of the interfaces gives a microscopic mechanism for the anomaleous behavior of the permeability in the transition region [4]. In the present paper we investigate theoretically how the interactive nature of absorbed foreign molecules affect the fluctuating interfaces and how this results in an impurity-induced modulation of the physical properties of the bilayer in the transition region. We argue that such lipid-mediated physical effects may be important for understanding the mechanisms of drug action.

In a previous paper, Jørgensen et al. [7] proposed a multi-state lattice model for the interaction of foreign molecules with phospholipid bilayers in the transition region. This model is based on a characterization of foreign molecules as either substitutional impurities, in which case they replace a lipid molecule, or as interstitial impurities, in which case they intercalate between the lipid acyl chains. The most important feature of the model is its ability to distinguish between certain foreign molecules whose concentration in the bilayer is fixed and others which partition between the membrane and various external gaseous and/or aqueous media. In the former case the model can be statistically and thermodynamically analysed in terms of a canonical ensemble whereas in the latter case a grand canonical ensemble employing a chemical potential is used to control the concentration of the foreign molecules in the membrane. It was pointed out [7] that it is extremely important for providing a proper interpretation of certain types of experiment on a given compound interacting with membranes to take the partitioning into account and to determine which ensemble is appropriate. In particular, determination of the proper statistical ensemble is necessary when changes (e.g., broadening) af a physical membrane quantity (e.g., specific heat or passive ion permability) are postulated as being due to the direct molecular interactions between the lipids and the drug or simply caused by the variation of an intrinsic thermodynamic variable (e.g., via exchange of drug molecules between the aqueous and membrane phases).

Jørgensen et al. [7] have examined the case when the interactions between interstitial foreign molecules and the lipid acyl-chain conformational states are *non-specific* in the sense that they depend only on the lipid-chain order parameter except for a global coupling constant. In the present paper we report a series of Monte Carlo computer simulations for lipid–foreign molecule systems using the model of Ref. 7 in the case where the interactions between the lipid chains and the foreign molecules are *specific* in the sense that the lipid–foreign molecule interactions depend explicitly on the excited gel-like conformational states of the acyl chains [8]. The principal result of the simulation is the discovery that foreign molecules can have a dramatic effect on the fluctuations in the transition region. The simulations presented in this paper were guided by the experimental results of Antunes-Madeira and Madeira [9–12] who measured the partition coefficients of a variety of drugs in phopholipid multilayer/water systems. These authors found that, for certain insecticides, such as parathion, lindane, DDT, and malathion [9–12], the partition coefficient exhibits a broad peak in the neighborhood of the main phase transition of the pure phospholipid membrane. We suggest that this behavior is related to the peaks observed in the passive ion permeability and partition coefficient for local anaesthetics such as cocain derivatives [13], as well as the characteristic broadening of the specific heat [14] and the increase in the passive ion permeability [15] observed for membranes interacting with general anaesthetics such as halothane. We contend that the model of Ref. 7 can be used to understand the nature

of these peaks in terms of the change in membrane density fluctuations caused by foreign molecules interacting with the lipid chains via specific interactions. Section 2 reviews the model of Ref. 7 and the numerical methods used and also gives a detailed discussion of the nature of the specific interactions. The results for the partition coefficient, the specific heat, the permeability, and the membrane area are presented in Section 3 for several sets of interaction parameters. It is found that specific interactions may lead to a peak in the partition coefficient. Section 3 also includes a description of the related heterogeneous membrane structures and the manner in which they are controlled by the foreign molecules. Section 4 contains a dicussion of the results in relation to experimental results and suggestions for further experimental and theoretical work. The conclusions are presented in Section 5.

2. Model and computer simulation techniques

The Hamiltonian for the model of Ref. 7 consists of three terms describing the pure lipid bilayer, the lipid–foreign molecule interactions, and the interactions between neighboring foreign molecules, respectively. The first term is basically the ten-state Pink model [16] which describes the main transition of saturated diacyl phospholipid bilayers in terms of acyl-chain conformational statistics and dispersion forces between chains. Each acyl chain is assigned a site in a triangular lattice and the translational degrees of freedom are ignored. Furthermore, the two monolayers of the bilayer membrane are considered as independent. The acyl-chain conformations are represented by ten single-chain states α, each described by a cross-sectional area A_α, an internal energy E_α, and an internal degeneracy D_α. The state $\alpha = 1$ corresponds to the all-*trans* conformation of the acyl chain and is characteristic of the gel phase. The state $\alpha = 10$ is averaged over D_{10} high-energy chain conformations characterized by many *gauche* rotations and is characteristic of the fluid phase. The eight intermediate states represent low-energy conformations whose selection is based on considerations of optimal chain packing and low conformational energy [16].

The second term in the model Hamiltonian is based on an extension of the hypothesis proposed de Verteuil et al. [17]. This extended hypothesis states that those foreign molecules, which do not change the transition enthalpy of the bilayer, intercalate between either the flexible acyl chains of the membrane or the polar heads of the lipid molecules and that their contribution to the exluded volume effect can be ignored [7]. The adsorbed foreign molecules can therefore be considered as interstitial impurities whose available locations are the centers of the triangles formed by three neighboring lipid acyl chains. The sites available to the foreign molecules therefore form a honeycomb lattice embedded in the triangular lattice formed by the acyl chains. The requirement that the lipid chains and the foreign molecules occupy separate lattices and hence cannot mix, results in the absence of an entropy of mixing. It is assumed that the interstitial impurities interact with the three neighboring lipid acyl chains as well as with other impurities occupying neighboring interstitial sites on the honeycomb lattice. The third term of the model Hamiltonian describes direct interactions between neighboring impurities on occupied interstitial sites.

The total Hamiltonian is written as follows

$$\mathcal{H} = \sum_i \sum_\alpha (E_\alpha + \Pi A_\alpha) \mathcal{L}_{i\alpha} - \frac{J_{LL}}{2} \sum_{i,j} \sum_{\alpha,\beta} I_\alpha I_\beta \mathcal{L}_{i\alpha} \mathcal{L}_{j\beta}$$

$$- \sum_i \sum_\alpha \sum_\ell J^\alpha_{LA} I_\alpha \mathcal{L}_{i\alpha} \mathcal{L}^A_\ell - \frac{J_{AA}}{2} \sum_{\ell,k} \mathcal{L}^A_\ell \mathcal{L}^A_k \qquad (1)$$

$\mathcal{L}_{i\alpha} = 0$ or 1 is an occupation variable for the conformational states of the chains and $\sum_\alpha \mathcal{L}_{i\alpha} = 1$. $\mathcal{L}^A_\ell$ is an interstitial site occupation variable which also takes on the values 0 or 1. I_α are nematic factors for the acyl-chain states [16] and J_{LL}, J^α_{LA}, and J_{AA} are interaction constants for the lipid–lipid, the lipid–foreign molecule and the foreign molecule–foreign molecule interactions, respectively. In the work reported in this paper, the lipid–foreign molecule interaction is taken to be specific, i.e., J^α_{LA} depends explicitly on the acyl-chain conformational state α. This is in contrast to the Ref. 7 in which J^α_{LA} was assumed to be independent of α for $\alpha = 1,2,\ldots,9$ and therefore to depend on the phase of the lipid membrane only, i.e., the lipid–foreign molecule interaction was assumed to be non-specific. Finally, $J_{LL} = 0.70985 \cdot 10^{-13}$ erg and $\Pi = 30$ dyn/cm as used in Refs. 7 and 18 for the case of a pure DPPC bilayer. The present model is therefore specified by the parameters J^α_{LA} ($\alpha = 1,2,\ldots 10$) and J_{AA}. The main phase transition temperature for pure DPPC bilayers is $T_m = 314$ K.

The concentration, x, of foreign molecules in the membrane is not conserved since partitioning between the membrane and the aqueous phase is assumed to occur. The use of a grand canonical ensemble is therefore appropriate and x is controlled by a chemical potential, μ. The effective Hamiltonian then becomes

$$\mathcal{H}_{\text{grand}} = \mathcal{H} - \mu \sum_\ell \mathcal{L}^A_\ell \qquad (2)$$

Since there are two acyl chains per lipid molecule, the natural concentration variable, x, is

$$x = \frac{2\langle \mathcal{L}^A_\ell \rangle}{1 + 2\langle \mathcal{L}^A_\ell \rangle} \qquad (3)$$

A possible motivation for introducing specific interactions between the interstitial foreign molecules and the various chain conformational states is given by the need to include steric effects in the formalism. In an environment of highly ordered acyl chains, the cost in van der Waals interaction energy between the lipid chains due to intercalation of the foreign molecules may well exceed the van der Waals interaction energy between the impurities and the lipids, so that the effective interactions between highly ordered lipids and the foreign molecules become repulsive. For the more disordered conformational states, the net interaction with the foreign molecules are similarly due to the interplay between van der Waals interactions and steric effects, implying that excited chain conformations give rise to more defects in the acyl-chain packing and the small foreign molecules can intercalate in between the chains and contribute to the interaction energy without significantly altering the interchain interactions.

The computer simulation techniques used in this paper are of the Monte Carlo type [19]. The simulations are performed on a finite triangular lattice of $N = L \cdot L$ sites for the acyl chains and a honeycomb lattice of $2N$ interstitial sites for the foreign molecules. Several different lattice sizes are used in order to estimate finite-size effects. Most of the simulation results reported below correspond to lattices with $N = 100 \cdot 100$ lipid chains, i.e., 5000 DPPC molecules in a monolayer. Toroidal boundary conditions are used, and all the lipid sites are occupied whereas the occupation of the honeycomb lattice is controlled by the chemical potential. The system is brought to equilibrium using Glauber dynamics for the single-chain conformational states and Kawasaki exchange dynamics for the lateral diffusion of the interstitial foreign molecules on the honeycomb lattice. Only the grand canonical ensemble is used in the simulations and the exchange of foreign molecules between the aqueous medium and the membrane is simulated by Glauber dynamics. A computer simulation approach, which takes full account of the thermal density fluctuations [19], permits an accurate determination of thermal expectation values. Furthermore, simulations give access to microconfigurations of the system which can be used to characterise phenomena such as membrane heterogenity and formation of lipid domains in the transition region.

3. Results

It was pointed out in Ref. 7 that models of lipid–foreign molecule interactions can be used to analyse experimental data for the variation of the membrane/water partition coefficient, $K(T)$, with temperature through the main transition. Such an analysis should then lead to a better understanding of the thermodynamic properties of lipid bilayer-water systems containing foreign molecules. The overall magnitude of K tells us which statistical ensemble is most suitable for the analysis. If K is small enough, i.e., if either the number of foreign molecules in the aqueous medium is much larger than in the membrane or K is much less than the ratio of the total volume of the aqueous medium to that of the membrane, it was shown in Ref. 7 that the grand canonical ensemble provides a complete description of the the partitioning of foreign molecules into the various lipid phases. In both situations the aqueous phase effectively acts as a reservoir of the foreign molecules and the reservoir can be described by a constant chemical potential.

In this section we present computer simulation results for $K(T)$ and the specific heat, $C_p(T)$, for two sets of model parameters characterised by specific interactions (Cases C and D below) and, for comparison, two different sets of model parameters characterised by non-specific interactions (Cases A and B below). In addition, a full analysis of the physical properties of the system is made for one of the two cases related to specific interactions. The limit of small K is applied in the calculations thus making K directly proportional to the concentration, x, of foreign molecules occupying interstitial sites in the bilayer model for the small temperature range under consideration. For a given μ, the concentration of foreign molecules in the aqueous phase is assumed to be constant over the temperature range considered. In the following we take $T_m(\mu)$ to denote the effective transition temperature as defined by the temperature at which the peak in the specific heat occurs for the given value of the chemical potential μ.

3.A. Non-specific interactions

Fig. 1 gives simulation results for $K(T) \simeq x(T)$ and $C_p(T)$ for the first set of model parameters (Case A) characterized by non-specific interactions between foreign molecules and lipid chains as reported in Ref. 7. In Case A, the interaction between chain states 1 to 9 and the interstitial foreign molecules is strongly repulsive implying strong mutual steric hindrance. A strong attractive interaction is used for the direct coupling between the foreign molecules. Fig. 1a shows that there is an abrupt change in x at the main phase transition temperature, indicating that the solubility of the impurities is considerably larger in the lipid fluid phase than in the gel phase. Fig. 1a also shows that the phase transition becomes more abrupt with increasing μ. This is signalled by both the the increase in sharpness of the jump dicontinuity in x and the suppression of the fluctuations in the transition region, i.e., the width of the transition peak in C_p is diminished (see Fig. 1b) and the peak itself becomes quite sharp. This phase behaviour which exhibits considerably different

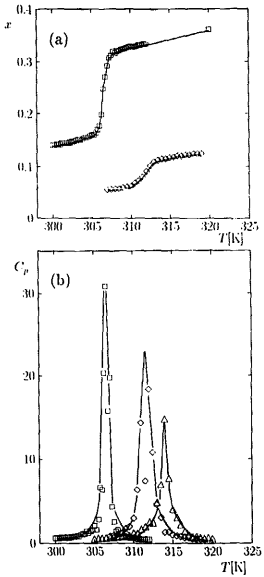

Fig. 1. Temperature dependence of the concentration x (a) and the specific heat C_p (b) in Case A of *non-specific* interactions between the foreign molecules and the lipids. The specific heat is in units of 10^{-14} erg K^{-1}. Results are shown for different values of the chemical potential, $\mu = -\infty$ (pure system) (△), -1.35 (◇) and $-0.94 \cdot 10^{-13}$ erg (□).

solubilities of the impurities in the variuos phases is characteristic of the model when strong attractive interactions are imposed between the interstitial foreign molecules. Fig. 2 gives simulation results for $x(T)$ and $C_p(T)$ for the second set of model parameters (Case B) with non-specific couplings between the foreign molecules and the chain conformational states. In Case B, the direct interactions between the interstitial foreign molecules are extremely weak in comparison with Case A. Fig. 2a shows that the solubilities of the impurities are more similar for the two lipid phases and consequently the jump in x (and therefore K) at T_m (μ) is smaller. Other signals of the phase transition are similar to those of the pure system. For example, Fig. 2b shows that C_p has a peak at T_m with pronounced wings away from T_m similar to the pure bilayer implying that strong long-range lateral density fluctuations in the lipid bilayer are still operative.

3.B. Specific interactions

Figs. 3 and 4 give simulation results for $x(T)$ and $C_p(T)$ for the two chosen sets of model parameters (Cases C and D) with specific couplings between the foreign molecules and the chain conformational states. In Case C corresponding to Fig. 3, the interactions between chain states 1 to 4 and the interstitial foreign molecules are again made strongly repulsive implying strong mutual steric hindrance. On the other hand, an attractive interaction is imposed between chain states 5 to 9 and the foreign molecules implying steric compatibility. The direct interaction between the foreign molecules is made relatively weak compared to Case A of Fig. 1. Fig. 3a shows that the partitioning of the foreign molecules for temperatures away from the transition region is similar to the non-specific case. However, in the transition region, the partition coefficient exhibits a definite fluctuation-induced peak below the phase transition temperature for high enough

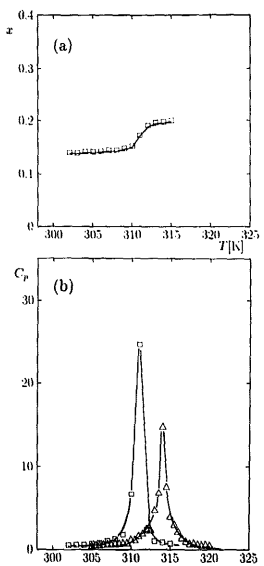

Fig. 2. Temperature dependence of the concentration x (a) and the specific heat C_p (b) in Case B of *non-specific* interactions between the foreign molecules and the lipids. The specific heat is in units of 10^{-14} erg K^{-1}. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△) and $-0.94 \cdot 10^{-13}$ erg (□).

μ. Fig. 3b shows that the specific heat peak broadens for the same value of μ but its maximum does not shift in temperature. The parameter set for Fig. 4, corresponding to Case D, is identical to that of Case C (Fig. 3) for the intermediate states but the interaction is made more repulsive between the all-*trans* state and the foreign molecules and more attractive between the 10th or fluid state and the foreign molecules. Fig. 4a shows the evolution of the peak in the partition coefficient as a function of μ. At low values of μ, the partition coefficient increases monotonically with increasing temperature in the gel phase. A cusp occurs at T_m after which the slope of the curve decreases considerably. As μ increases, the cusp is replaced by a broad peak with its maximum lying below T_m. Fig. 4b gives the corresponding variation of C_P with temperature. As μ increases, the peak in the specific heat, $C_P(T)$, in the transition region broadens and the position of the maximum decreases and shifts to lower teperatures. Fig. 4b also shows that this shift in transition temperature eventually saturates. The transition enthalpy as measured by the integral of $C_P(T)$ over T is nearly independent of μ as signalled by the broadening of the peak and its concomitant decrease in intensity. It is important to examine the system corresponding to Fig.

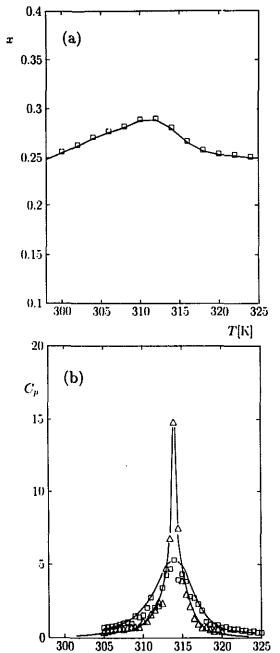

Fig. 3. Temperature dependence of the concentration x (a) and the specific heat C_P (b) in Case C of *specific* interactions between the foreign molecules and the lipids. The specific heat is in units of 10^{-14} erg K^{-1}. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△) and $-0.94 \cdot 10^{-13}$ erg (□).

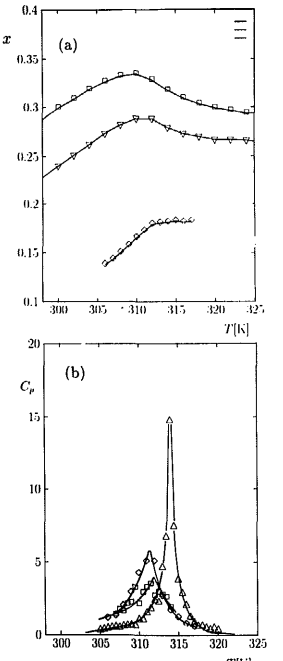

Fig. 4. Temperature dependence of the concentration x (a) and the specific heat C_P (b) in Case D of *specific* interactions between the foreign molecules and the lipids. The specific heat is in units of 10^{-14} erg K^{-1}. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△), -1.20 (◇), -1.00 (▽), and $-0.94 \cdot 10^{-13}$ erg (□).

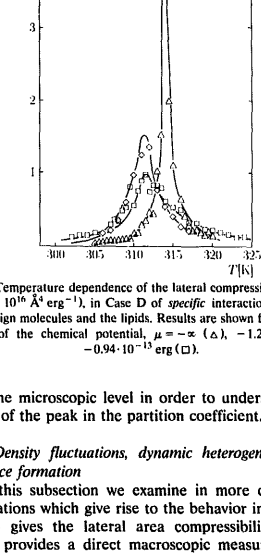

Fig. 5. Temperature dependence of the lateral compressibility, χ (in units of 10^{16} Å^4 erg^{-1}), in Case D of *specific* interactions between the foreign molecules and the lipids. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△), -1.20 (◇), and $-0.94 \cdot 10^{-13}$ erg (□).

4 at the microscopic level in order to understand the origin of the peak in the partition coefficient.

3.C. Density fluctuations, dynamic heterogeneity, and interface formation

In this subsection we examine in more detail the fluctuations which give rise to the behavior in Case D. Fig. 5 gives the lateral area compressibility, $\chi(T)$, which provides a direct macroscopic measure of the lateral density fluctuations in the system. This figure shows that the presence of foreign molecules in the bilayer has a considerable effect on $\chi(T)$. This can be seen in Fig. 5 which shows that the maximum value of $\chi(T)$ at $T_m(\mu)$ is considerably reduced, while the fluctuations away from $T_m(\mu)$ are enhanced, especially for temperatures below the transition temperature. The fluctuations in the transition region are related to the formation of fluctuating lipid domains in the bulk membrane matrix. This is illustrated in Fig. 6 which shows for both a pure lipid bilayer and a bilayer containing foreign molecules that fluid domains are formed in the gel phase below the transition and gel domains are formed in the fluid phase above the transition. As a consequence, the membrane is characterized on the mesoscopic level by an equilibrium domain-size distribution function from which the average domain size, $\langle \ell(T,\mu) \rangle$ can be derived. For a pure bilayer, $\langle \ell \rangle$ increases sharply as the transition region is approached from either phase, as shown in Fig. 7. This figure also shows that the presence of foreign molecules in the bilayer results in a decrease in $\langle \ell \rangle$ at the transition temperature, while $\langle \ell \rangle$ increases away from the transition, particularly in the gel phase. Fig. 7 also indicates that the temperature dependences of $\langle \ell \rangle$ and χ are similar. The observations made for the average property $\langle \ell \rangle$ is substantiated by inspection of the instantaneous snapshots of the characteristic microconfigurations in Fig. 6.

The formation of lipid domains in the transition region implies a dynamic phenomenon involving a set of fluctuating random interfaces between the domains and the bulk. We refer to this phenomenon as dynamic membrane heterogeneity. The snapshots in Fig. 6 show in accordance with the data in Fig. 7 that the foreign molecules make the heterogenous membrane states prevail over a larger temperature range. Since the interfaces have very special packing characteristics dominated by excited lipid-chain conformations [4,20], the interfaces are expected to act as sinks for defects and impurities. In fact, a closer analysis of the lateral distribution of the foreign molecules in configurations like the ones in Fig. 6 shows that this distribution is very heterogeneous with a dramatic accumulation of the foreign molecules in the interfaces. This is quantitatively demonstrated in Fig. 8a which shows the local impurity concentrations for the three membrane regions: the bulk, the domains (clusters), and the domain interfaces. These local concentrations, c_b, c_c, and c_i, are defined as the average number of foreign molecules per lipid molecule in the pertinent region of the membrane (i.e., $0 \leqslant c_b, c_c, c_i \leqslant 2$). Simultaneously with the accumulation in the interfaces, the foreign molecules change the molecular structure of the interface by inducing more excited, intermediate conformational states of the acyl chains. This effect is illustrated in Fig. 8b where W denotes the relative occurrence of intermediate conformational states in the interfacial region. Obviously, these changes in the interfacial region are related to the enhancement of the macroscopic fluctuations due to the specific interactions between the adsorbed foreign molecules and the intermediate gel conformations of the acyl chains.

3.D. Passive trans-membrane permeability

It is possible via a simple model assumption to relate the formation of interfacial regions in the membrane to passive trans-membrane permeation by ions [4,20] for example. According to this assumption, different regional probabilities of transfer, p_b, p_c, and p_i, are associated with the three different regions of the fluctuating membrane structure. The extent of these regions is determined by the fractional areas, $a_b(x,T)$, $a_c(x,T)$, and $a_i(x,T)$, into which the total membrane area, $A(x,T)$, can be devided. Furthermore, it is assumed that the regional probabilities of transfer are independent of temperature and that the transfer predominantly takes place in the interface, i.e., $p_i \gg p_b$, p_c. This implies that the temperature dependence of

the permeability is determined mainly by the temperature dependence of the fractional areas. Using these quantities, a relative permeability, $R(x,T)$, may be obtained as [20]

$$R(x,T) = A(x,T)^{-1/2}T^{1/2}[a_b(x,T)p_b + a_c(x,T)p_c + a_i(x,T)p_i] \quad (4)$$

It is shown in Ref. 20 that $R(x,T)$ is proportional to the logarithm of the fraction of molecules which would be retained in a liposome during a permeation experiment [20]. It should be noted that the above model and formalism are quite general and should apply to permeation of ions as well as of a variety of different molecular species [4]. We shall use this theory below in connection with the simulation data of the present paper, to predict the relative permeability for Na^+ ions. The regional probabilities of transfer are assumed to have the same values as determined in Ref. 4.

The data for the membrane area per lipid chain, $A(x,T)$, and the fractional areas, $a_h(x,T)$, $a_c(x,T)$, and

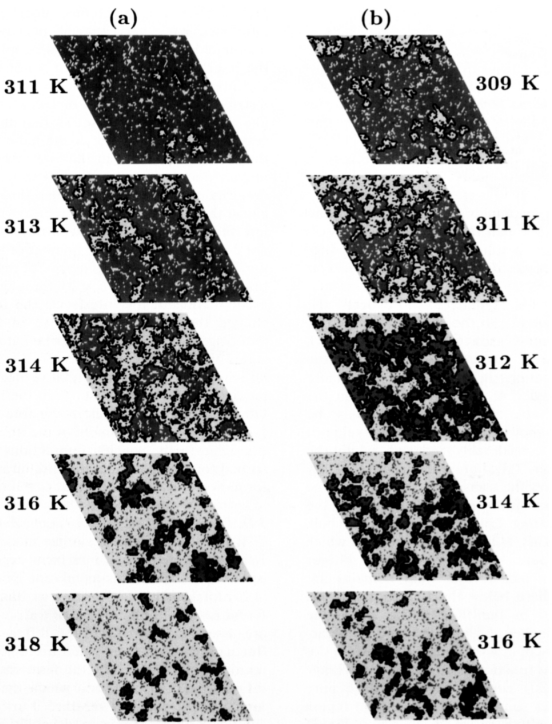

Fig. 6. Snapshots of microconfigurations characteristic of different temperatures in the neighborhood of the lipid bilayer phase transition transition. The data refer to Case D of *specific* interactions between the foreign molecules and the lipids. (a): the pure lipid bilayer ($\mu = -\infty$) and (b): the lipid bilayer in the presence of foreign molecules governed by a chemical potential $\mu = -1.20 \cdot 10^{-13}$ erg. The interfaces between the lipid domains and the bulk are highlighted in black, whereas grey and light regions correspond to gel and fluid regions, respectively.

$a_i(x,T)$, required to calculate the relative permeability, $R(x,T)$ in Eqn. 4, are given in Figs. 9 and 10, respectively, for Case D. These figures show that the presence of foreign molecules in the bilayer leads to a dramatic increase in the fractional membrane area associated with the interfacial region. This is clearly reflected in the permeability data corresponding to Figs. 9 and 10 as can be seen in Fig. 11. This figure shows that the foreign molecules induce a significant increase in the permeability by making the bilayer much more leaky over a broader temperature range.

4. Comparison with experiment

In the previous section we described the results of computer simulations for a theoretical model with specific interactions between foreign molecules and acyl-chain conformational states. In this section, we use these results for the interpretation of experimental data for three groups of compounds in the neighborhood of the main phase transition. The first group consists of gaseous general anaesthetics represented by halothane. We shall only discuss a few experimental results in the case of general anaesthetics since this is a very large group of compounds on which a substantial body of different experimental data exists. The other two groups are water-soluble compounds represented by cocaine derivatives (local anaesthetics), such as procaine, and organochlorine insecticides, such as lindane. The last two groups of compounds are structurally very different from each other. The cocaine derivatives usually interact with the bilayer near the hydrocabon water interface [21,22] while the insecticides are believed to be buried deeply in the hydrocarbon core of the acyl chains [10,23]. Nevertheless, both molecular groups affect the physical properties of the lipid bilayer in the transition region in a similar manner. Finally, both lindane [10] and procaine [13] have a small DPPC-bilayer/water partition coefficient of order 100 at temperatures away from T_m. A grand canonical decription is therefore appropriate for most experiments on these systems.

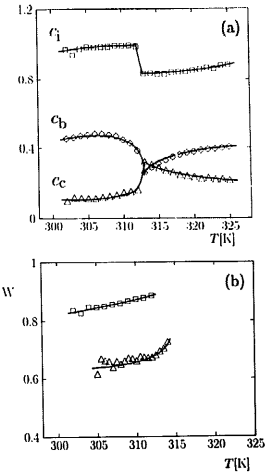

Fig. 8. (a): Local concentrations, c_b, c_c, and c_i, of foreign molecules in the three different parts of the bilayer, the bulk (b), the lipid domains or clusters (c), and in interfaces (i) for $\mu = -0.94 \cdot 10^{-13}$ erg. (b): Relative occurrence, W, of intermediate conformational states of the acyl chains in the interfacial region of the bilayer for a pure bilayer (△) and a bilayer with foreign molecules in a concentration corresponding to $\mu = -0.94 \cdot 10^{-13}$ erg. The data in both (a) and (b) correspond to Case D of *specific* interactions between the foreign molecules and the lipids.

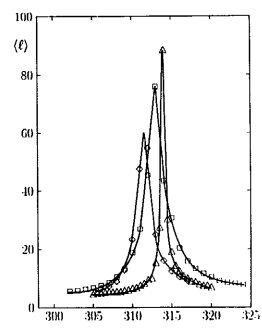

Fig. 7. Temperature dependence of the average lipid domain size, $\langle \ell \rangle$ (in units of number of acyl chains) in Case D of *specific* interactions between the foreign molecules and the lipids. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△), -1.20 (◇), and $-0.94 \cdot 10^{-13}$ erg (□).

The presence of cocaine derivatives in the bilayer/water system reduces $T_m(\mu)$ [24,25]. The peak height of the specific heat at T_m is also reduced and the peak itself is broadened thereby keeping the total enthalpy change constant as more anaesthetics is added to the system. An example of the effect of insecticides on the transition temperature is provided by lindane. This compound causes a decrease in T_m, implying a stronger affinity of lindane for the fluid phase than for the gel phase. Lindane only weakly influences the acyl-chain order in the fluid phase, whereas the chain

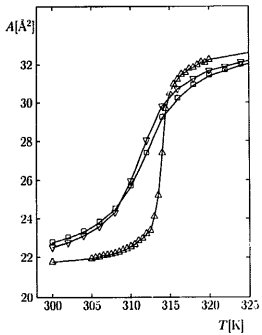

Fig. 9. Temperature dependence of the average lipid membrane area, $A(T)$, in Case D of *specific* interactions between the foreign molecules and the lipids. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△), -1.00 (▽), and $-0.94 \cdot 10^{-13}$ (□).

order is strongly perturbed in the gel phase [26]. This behaviour is indeed reproduced by the present model as shown in Fig. 9, recalling that the membrane area is inversely related to the average acyl-chain order [27]. Measurements of the changes in the optical properties of DMPC-bilayers in the presence of procaine point in the same direction [15]. The effects of DDT [28] on the transition properties are very similar to those of lindane. For example, the transition enthalpy does not vary significantly with concentration [29] and the acyl-chain order is mainly affected in the gel phase [30]. In the case of halothane, the experimental data [14] for C_P are very similar to those shown in Fig. 4 with a downward shift of the transition temperature and a concomitant substantial broadening of the specific heat peak. At the same time the peak intensity at the transition is suppressed.

The calculated partition coefficients shown in Figs. 3 and 4 display a maximum in the transition region. Unfortunately, experimental results for the membrane/water partition coefficients of bilayer/water systems containing anaesthetics and insecticides are very sparse with few data points in the transition region. However, the available data for the cocain derivatives (dibucaine, tetracaine, procaine, and benzocaine) [13] and the insecticides (DDT, lindane, parathion, and malathion) [9–12] clearly show that $K(T)$ has a maximum close to T_m.

The passive trans-membrane permeability of pure DPPC bilayers displays a maximum at T_m, for cations for example [31]. For bilayers containing local anaesthetics, the permeability is generally enhanced with an overall temperature dependence similar to the pure case [13]. These experimental facts are in accordance with the predictions of the model of the present paper as shown in Fig. 11. For example procaine strongly enhances the Na^+-ion permeability of DMPC bilayers, especially for temperatures below T_m [15]. A systematic study of the ANS-permeability of DMPC-bilayers shows similar effects [15]. Detailed studies of the temperature dependence of the permeability of lipid bilayers in presence of lindane have not been carried out yet. However, it is demonstrated in Ref. 32 that lindane as well as other insecticides increase the permeability of many-component lipid bilayers. The effect of procaine on the permeability of DPPC bilayers has been meas-

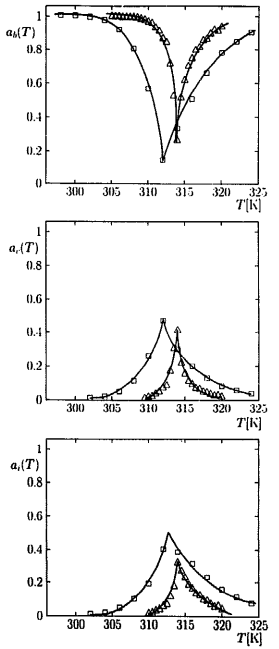

Fig. 10. Temperature dependence of the fractional membrane areas, a_b, a_c and a_i, corresponding to the bulk, the domain (cluster), and the interfacial regions. in Case D of *specific* interactions between the foreign molecules and the lipids. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△) and $-0.94 \cdot 10^{-13}$ erg (□).

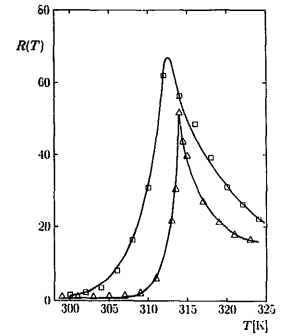

Fig. 11. Temperature dependence of realtive passive transmembrane permeability, $R(T)$ in Eqn. 4, of Na^+-ions in Case D of *specific* interactions between the foreign molecules and the lipids. Results are shown for different values of the chemical potential, $\mu = -\infty$ (△) and $-0.94 \cdot 10^{-13}$ erg (□).

ured [15] and it was found that the permeability increased as more procain is incorporated. Finally, it has been observed that the general anaesthetic halothane also tends to increase bilayer permeability [33].

Another bilayer phenomena which is related to the fluctuations and the dynamics of the lipid matrix is the diffusivity of the foreign molecules. Measurements of the lateral diffusion of lindane in DPPC bilayers [34] show that the effective diffusion constant displays a minimum close to T_m. This result can be interpreted on the basis of our simulation results as follows. At T_m, a substantial part of the foreign molecules are located in the lipid-domain interfaces which act as dynamic pinning sites for the foreign molecules. The foreign molecules therefore spend a considerable time in the lipid-domain interfaces, where their motion is limited by slow interface dynamics and the one-dimensional nature of the interfaces.

5. Conclusions

We have presented results from a computer simulation study of a simple microscopic interaction model for the description of the cooperative phenomena associated with the lipid bilayer chain-melting transition in the presence of water-soluble foreign molecules. The interactions between the foreign molecules and the lipid acyl chains were taken to be specific with respect to the acyl-chain conformational states. The model does not give a full representation of the molecular details of the interactions between the lipid membrane and the foreign molecules, but it is capable of describing some general and characteristic features of the cooperative phenomena, which are reflected in measurable thermal quantities. Furthermore, the model is capable of providing information on both the microscopic membrane structure and lateral organization and on the manner in which these properties are altered by the presence of the foreign molecules.

The most important result of our study is the discovery of a remarkable correlation between the lateral density fluctuations in the lipid bilayer and the presence of the interstitial foreign molecules which have specific interactions with the lipid acyl chains. Macroscopically this leads to a significant enhancement of response functions like the specific heat, the lateral compressibility, and certain derived quantities, such as the passive ion permeability, for temperatures away from the transition point. Microscopically, we find that the density fluctuations manifest themselves in the formation of dynamically heterogeneous membrane structures which are significantly altered by the presence of the foreign molecules. Specifically we have shown that the foreign molecules accumulate in the interfacial regions of the heterogenous membrane structure leading to regions of very high local concentration. These phenomena are most clearly reflected in a non-classical peak of the partition coefficient at the transition temperature. This is an example of a quite general physical phenomenon in cooperatively interacting molecular systems known as inhanced adsorption which plays an important role in phenomena such as heterogeneous catalysis [35].

The fluctuation-induced peaks in the partition coefficient should be contrasted to the more classical behavior of a jump-discontinuity in the partition coefficient at the transition for drugs which are highly water-soluble but at the same time has a sharp specific heat peak. This is the case for compounds like the antidepressant chlorpromazine [36,7] and possible also for adriamycin derivatives [37]. However, as we have pointed out in an earlier paper [7], the experimental situation is in many cases quite unsatisfactory due to the lack of systematic studies of the temperature dependence of the partition coefficient.

The model of the present paper hence describes the subtle interplay between lateral density fluctuations and the binding of foreign molecules to interstial sites in the bilayer membrane. The strong coupling between the binding of foreign molecules and lateral density fluctuations suggested by the computer simulations is difficult to directly verify experimentally. However, experimental results on some insecticides show that the peak in the partition coefficient at T_m sharpens as the lipid chain length increases [9–12]. Since this behavior is similar to the effect of chain length on the lateral density fluctuations [38], this strongly suggests the existence of such a coupling. The available data for the

partition coefficient of cocaine derivatives [13] has a weak dependence on acyl-chain length but the trend is the same as for the insecticides.

We have pointed out that the coupling between the lateral density fluctuations and the physical effects on lipid membranes of a variety of molecular compounds interacting with membranes may explain a large body of experimental observations on several different classes of compounds, including general and local anaesthetics and certain insecticides. It is likely that this type of coupling also may explain similar effects observed for some alcohols [39] and herbicides [40]. We wish to point out that the finding of fluctuation-induced broadening of physical quantities such as the specific heat and the enhancement of permeabilities are basic consequences of the cooperative nature of the system and its specific interactions. This statement underscores our earlier assertion that a proper identification of the thermodynamic variables are crucial for the interpretation of experiments on compounds which partition between the membrane and the aqueous phase. As an example, our results suggest that the experimental finding of a broadened specific heat for halothane, which has led to substantial discussion in the literature, is more naturally understood in terms of density fluctuations than as a consequence of non-equilibrium or finite-system thermodynamics [41].

Our simulation results give an indication of how foreign molecules change the heterogeneity of lipid membranes. This substantiates what was surmised earlier by a number of workers who related the action of drugs on lipid membranes to the formation of different defect structures [15]. This brings us back to one of the main motivations for studying the physical effects of drug on membranes which is related to the ongoing discussion of the possible mechanisms of action of pharmacological substances. We suggest that the results presented in this paper give support to the hypothesis that the action of drugs may be lipid mediated [42] and specifically controlled by the tendency for induced dynamical heterogeneity. The accumulation of the drug molecules in the interfacial region, which is in turn enhanced by the presence of the drug molecules, gives the drug molecules themselves more direct access to many proteins which also have a tendency to accumulate along lines of defects [43]. Our results should therefore be of interest in relation to the current discussion of the influence of drugs and insecticides on protein function via a change of the lipid environment in the neighborhood of the protein [44–49].

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