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ARTICLE

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A theoretical model for the association of amphiphilic transmembrane peptides in lipid bilayers

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Abstract A theoretical model is proposed for the association of trans-bilayer peptides in lipid bilayers. The model is based on a lattice model for the pure lipid bilayer, which accounts accurately for the most important conformational states of the lipids and their mutual interactions and statistics. Within the lattice formulation the bilayer is formed by two independent monolayers, each represented by a triangular lattice, on which sites the lipid chains are arrayed. The peptides are represented by regular objects, with no internal flexibility, and with a projected area on the bilayer plane corresponding to a hexagon with seven lattice sites. In addition, it is assumed that each peptide surface at the interface with the lipid chains is partially hydrophilic, and therefore interacts with the surrounding lipid matrix via selective anisotropic forces. The peptides would therefore assemble in order to shield their hydrophilic residues from the hydrophobic surroundings. The model describes the self-association of peptides in lipid bilayers via lateral and rotational diffusion, anisotropic lipid-peptide interactions, and peptide-peptide interactions involving the peptide hydrophilic regions. The intent of this model study is to analyse the conditions under which the association of trans-bilayer and partially hydrophilic peptides (or their dispersion in the lipid matrix) is lipid-mediated, and to what extent it is induced by direct interactions between the hydrophilic regions of the peptides. The model properties are calculated by a Monte Carlo computer simulation technique within the canonical ensemble. The results from the model study indicate that direct interactions between the hydrophilic regions of the peptides are necessary to induce peptide association in the lipid bilayer in the fluid phase. Furthermore, peptides within each aggregate are oriented in such a way as to shield their hydrophilic regions from the hydrophobic environment. The average number of peptides present in the aggregates formed depends on the degree of mismatch between the peptide hydrophobic length and the lipid bilayer hydrophobic thickness: The lower the degree of mismatch is the higher this number is.

Key words Lipid-peptide interaction · DPPC · Hydrophobic matching · Peptide aggregation · Monte Carlo simulation

Abbreviations DPPC dipalmytoylphosphatidylcholine

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Introduction

Many integral membrane proteins consist of transmembrane α-helical peptides which associate in bundles (von Heijne and Manoil 1990). Among the few integral membrane proteins whose transmembrane secondary structure is established at high resolution, bacteriorhodopsin is an example of a bundle structure which is made by the association of seven α-helices (Henderson and Unwin 1975). More recently, high resolution structures have also been established for other helix-bundle proteins: The plant light-harvesting complex (Külbrandt and Wang 1991), the photosystem I reaction center (Kraus et al. 1993), and rhodopsin (Schertler et al. 1993). Helix association and bundle formation are also involved in the formation of membrane channels: An example is alamethicin, an amphiphilic helical peptide of fungal origin, which associates and forms aqueous pores made of eight alamethicin monomers (He et al. 1995); Another example concerns the transmembrane α-helices of phospholamdan, which aggregate in pentamers and thus form a cardiac ion channel in the membrane of the sarcoplasmic reticulum (Arkin et al. 1994; Ludlam et al. 1996). Proteins with the helix bundle motif are found in the inner membrane of bacteria as well as in most membrane systems of eukaryotic cells and are therefore involved in numerous membrane functions (see reviews by

Lemmon and Engelman 1994; Shai 1995) connected with receptor activity (Clapham 1996), permeability, maintainance of lipid asymmetry in the two leaflets of the bilayer (Fattal et al. 1994), antimicrobial activity (He et al. 1995; Gazit et al. 1995), transmembrane signaling, and protein sorting for the different cell compartments. A better knowledge of the energetics involved in peptide association in membranes is thus important for a better understanding of many biological functions.

Structural studies and sequence analysis of α-helices in protein aggregates, such as bundles or channels, indicate that the residues exposed to the lipid environment are more hydrophobic than those buried in the interior of the bundles, which face the buried sides of other helices (Rees et al. 1989). Therefore, after incorporation into the lipid bilayers, and via lateral diffusion, partially hydrophobic α-helices would assemble into molecular aggregates in order to shield their polar residues from the hydrophobic environment. However, the forces which are responsible for the self-association and stability of the helices in the highly hydrophobic lipid environment are still a matter of debate (Lemmon and Engelman 1992; Haltia and Freire 1995); peptide assemblies are most probably induced by non-specific peptide-peptide interactions, peptide-lipid interactions and peptide-water interactions (in the case of peptides forming ion-channels). Several experimental observations indicate that electrostatic interactions also play a stabilizing role in the assembly of oligomeric membrane proteins (Cosson et al. 1991; Arkin et al. 1996), as well as in water-soluble proteins (Fairman et al. 1996). In general, charged residues in proteins seem to provide the topological information which is necessary for the suitable organization of the proteins in membranes (von Heijne et al. 1988). Furthermore, the stable association of transmembrane peptides in the membranes also seems to depend on the detailed packing between the residues of the peptides, as suggested recently for phospholamdan (Engelman et al. 1996). A number of experiments have actually shown that synthetic α-helices derived from the transmembrane domains of proteins can assemble and substitute for the native aggregates as well as their functions. The proteins which have been used for these reconstitution experiments are bacteriorhodopsin (Kahn and Engelman 1992), glycophorin (Bormann et al. 1989), and, more recently, phospholamdan (Arkin et al. 1994). Popot and Engelman (Popot and Engelman 1990; Popot 1993) rationalized the results from the early reconstitution experiments within the framework of a two-stage model, where the possible factors leading to the formation of helix assembly were qualitatively explained. According to the two stage model, the α-helices are first independently folded and inserted into the lipid bilayer (first stage); it is only during a second stage that the helices interact and assemble to form the transmembrane structures.

From a theoretical point of view, Wang and Pullman (1991) tried to quantify those factors which lead to the formation of aggregates of α-helices such as bundle proteins. They analysed the stability of different aggregates of hydrophobic polyalanine helices and lipid acyl chains by calculating the dissociation energy of the molecules in a given aggregate. They found that the hydrophobic helices prefer to pack together rather than remain dispersed in the lipid matrix and they attributed this preference to the strength of the peptide-peptide interaction. However, their conclusions were based on the results obtained from calculations involving only enthalpic contributions to the dissociation energy, while possible entropic contributions were neglected. Based on the results of a three-dimensional molecular hydrophobicity potential calculation, Brasseur (1991) estimated the mode of interaction and assembly of partially hydrophobic helices in the phospholipid bilayer and proposed a general system for classifying the several types of helical peptides which one finds in nature, such as ion-channel forming helices, receptor protein helices, fusiogenic helices, or amphiphilic non trans-membrane helices. According to the theoretical predictions of this author, helices with a predominantly hydrophobic surface can assemble into bundles whereas more hydrophilic helices prefer to stay at the water-bilayer interface. The results are based on a single helix calculation, no explicit lipid-peptide interactions are considered, and no conformational and statistical properties of the lipid molecules are taken into account. These calculations cannot predict the lateral organization of the lipid-peptide mixtures, since such organization is a result of the cooperative behaviour of a many-body system. The energetics of helix association into aggregates have recently been studied by Ben-Tal and Honig (1996), who made use of a phenomenological theory to calculate the electrostatic interaction free energy between two α-helices in a simple representation of a lipid bilayer. Their results suggest that helix aggregation is not due to non-specific effects, but is rather due to specific interactions involving, for example, the formation of hydrogen bonds between side chains on different helices.

Few statistical mechanical lattice models have been used to investigated the lateral organization of lipid-peptide systems (Sperotto and Mouritsen 1991; and references therein). More recently, Heimburg and Biltonen (1996), have proposed a lipid-protein interaction model to study the lateral distribution of peripheral and integral membrane proteins in lipid bilayers. The lipid-protein interactions are described in terms of binding energy differences upon protein binding to gel or fluid lipids. The work focuses mainly on the interpretation of heat capacity functions in terms of protein aggregation state, as well as in terms of the difference in phase behaviour between lipid-protein mixtures involving peripheral or integral proteins.

The lattice models just mentioned consider integral membrane proteins with a surface in contact with the bilayers lipid chains which is fully hydrophobic. In the present paper a theoretical lattice model is proposed for the association of partially polar, or fully hydrophobic, transmembrane peptides in lipid bilayers. The model is based on a microscopic lattice model for the pure lipid bilayer (Pink et al. 1980). The lattice formulation accurately accounts for the conformational states of the lipid chains and their mutual interaction and statistics, but it suppresses the translational modes of the lipids and confines their glyce-

rol backbone to a plane. The Pink model has been adopted in the past to study the cooperative phase behaviour of lipid-protein mixtures (Lookman et al. 1982; Pink 1984) using a set of lipid-protein interaction constants. Here, as well as in a previous model (Sperotto and Mouritsen 1991) an attempt is made to identify part of the lipid-peptide interactions in terms of molecular properties. According to the model formulation the peptides are all assumed to be identical and are treated as rigid objects with a cylindrical shape characterized by a lateral surface which can be hydrophobic or partially hydrophilic, as shown schematically in Fig. 1. In the latter case it is assumed that, even though the peptides can be incorporated into the lipid bilayer, their hydrophobic sequence contains some polar residues which prefer to be in contact with a polar environment rather than the strongly non-polar environment of the hydrocarbon lipid chains. In fact, polar residues may form hydrogen bonds with water (Meyer 1992), or with polar residues from other peptides (Stickle et al. 1992) thus reducing their polarity (Chothia 1976) and making them more stable than in the non-polar environment. The direct interaction between the lipids and the peptide, and between the hydrophilic surface of the peptides, is assumed to be short range and to include terms related to the hydrophobic lipid-peptide interfacial contacts, and to the hydrophilic peptide-peptide contacts. It also includes a term which accounts for the possible mismatch between the lipid bilayer and peptide hydrophobic lengths, as well as a term which is related to the interaction of the peptide polar surface with the water. Direct van der Waals-like interactions between the hydrophobic regions of the peptides are not taken into consideration; by focusing on a model without this type of interaction, one is able to isolate the effect of purely lipid-mediated peptide aggregation.

The paper presents the results of a systematic model study of the lateral distribution of peptides in fluid dipalmitoylphosphatidylcholine (DPPC) bilayers. The different lipid-peptide and peptide-peptide interaction parameters which have been considered were used to test the model and also to gain insight into the basic physical mechanisms behind the lateral organization of α-helical peptides in lipid bilayers. In particular, the intent with this model study was to analyse the conditions under which the assembly of trans-bilayer and partially hydrophilic helices (or their dispersion in the lipid matrix) is lipid-mediated, and to what extent it may be induced by direct interactions between the hydrophilic regions of the helices.

The model properties were calculated by Monte Carlo computer simulation techniques within the canonical ensemble (Mouritsen 1990), which treat the entropy of mixing correctly. The advantage of using these simulation techniques is that it is possible to treat a sufficiently large number of molecules, and therefore to account correctly for the system cooperativity. Furthermore, it is possible to inspect the bilayer configuration on the molecular level, and hence to determine the state of peptide assembly.

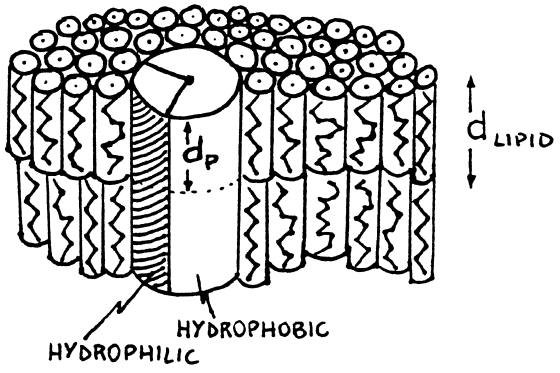

Fig. 1 Schematic illustration of a hydrophobically anisotropic transmembrane peptide embedded in a lipid bilayer

Theory

A statistical mechanical lattice model is proposed for the association of identical amphiphilic peptides in a lipid bilayer. The model, which is a modification of a microscopic model used to describe a lipid bilayer incorporated with small hydrophobic peptides (Sperotto and Mouritsen 1991), is built on the ten-state Pink model (Pink et al. 1980) used to describe the gel-fluid transition of the lipids in the fully hydrated state. This a pseudo-two-dimensional lattice model which neglects the translational modes of the lipid molecules and focuses on the conformational degrees of freedom of the acyl chains. Within the Pink model, the bilayer is formed by two independent monolayers, each represented by a triangular lattice, on whose sites the lipid chains are arrayed. Each chain can take on one of ten conformational states, m, each of which is characterized by an internal energy E_m, a hydrocarbon chain length d_m, and a degeneracy D_m, accounting for the number of chain conformations described by the same energy E_m and the same projected area, A_m, on the underlying lattice. The ten states are derived from the all-*trans* state by means of *trans*-gauche isomerism. The state $m = 1$ is the non-degenerate gel-like state, while the state $m = 10$ is the highly degenerate state of the fluid phase. In the case of DPPC, the corresponding values of the acyl chain lengths are 18.75 Å and 11.25 Å, respectively. The eight-intermediate states are gel-like states.

The Hamiltonian energy $H_{\mathrm{L-L}}$ for the pure lipid bilayer is then expressed as:

$$H_{\mathrm{L-L}} = \sum_i \sum_m (E_m + \Pi A_m) L_{m,i} - \frac{J_0}{2} \sum_{\langle i,j \rangle} \sum_{m,n} I(d_m, d_n) L_{m,i} L_{n,j}, \quad (1)$$

where Π is a lateral pressure, and J_0 is the strength of the van der Waals-like interaction between neighboring chains, $I(d_m, d_n)$ is an interaction matrix which involves both distance and shape dependence, and $L_{m,i}$ are site occupation variables: $L_{m,i} = 1$ if the chain on site i is in state m, otherwise $L_{m,i} = 0$. The model parameters Π and J_0 were chosen in order to reproduce the transition temperature

$T_m = 314$ K for the pure DPPC bilayer: $\Pi = 30$ dyne/cm, $J_0 = 0.70985 \times 10^{-13}$ erg (Mouritsen et al. 1983).

Within the lattice formulation, the peptides are represented by regular objects, with no appreciable internal flexibility, and with a projected area on the bilayer plane corresponding to a hexagon with seven lattice sites (the peptide-center site plus its six nearest neighbours). The bilayer spanning part of each helix is assumed to be smooth, so that it can be characterized by a cross-sectional area, A_P, and a hydrophobic core length, $2 \times d_P$. The value of A_P has been chosen in order to model a size corresponding approximately to that of an α-helix, i.e. $A_P = 7 \times A_1$, where $A_1 = 20.4$ Å^2.

In addition, one third of the peptide surface (in the lattice formulation, this corresponds to two of the six hexagon sites) at the interface with the lipid chains may have a different hydrophobicity compared to the rest of the surface, as shown schematically in Fig. 1. The idea is to model α-helices which in their trans-membrane sequence have polar residues intercalated between three or four non-polar residues. In the following, the suffix 'ϕ' is used to denote the two hexagon sites at the interface with the lipid chains corresponding to the polar region of each helix. The peptides are allowed to rotate and diffuse laterally in the lipid bilayer plane, and to interact with one another and with the lipid matrix via selective *anisotropic* forces.

The lipid-peptide interactions referring to the hydrophobic region of the peptides have been incorporated into the model in Eq. (1) in the spirit of the phenomenological *mattress model* of lipid-protein interactions in membranes (Mouritsen and Bloom 1984; Sperotto and Mouritsen 1991). This model is formulated in terms of an attractive van der Waals-like interaction and a repulsive *mismatch* interaction which reflects the possible incompatibility between the acyl chain length and the peptide hydrophobic length. The formulation of the lipid-peptide interaction in terms of a mismatch parameter has already proven to be useful in the interpretation of a number of experimental results for lipid-protein mixtures (Sperotto and Mouritsen 1988, 1991; Sperotto et al. 1989; Zhang et al. 1993). The Hamiltonian function for these interactions can then be written as:

$$H_{\mathrm{L-P}} = \Pi \left(\frac{A_P}{7} \right) \sum_i L_i + H^{vdW}_{\mathrm{L-P}} + \begin{cases} \dfrac{(2\gamma)}{2} \displaystyle\sum_{\langle i,j \rangle} \sum_m |d_{m,i} - d_P| L_{m,i} L_j, & \text{if } d_P < d_{m,i} \\ \uparrow \textit{ repulsive } \uparrow & \\ \downarrow \textit{ repulsive } \downarrow & \\ \dfrac{\gamma}{2} \displaystyle\sum_{\langle i,j \rangle} \sum_m |d_{m,i} - d_P| L_{m,i} L_j, & \text{if } d_P > d_{m,i} \end{cases} \tag{2}$$

where

$$H^{vdW}_{\mathrm{L-P}} = -\frac{\nu}{2} \sum_{\langle i,j \rangle} \sum_m \min(d_{m,i}, d_P) L_{m,i} L_j, \qquad \uparrow \textit{ attractive } \uparrow \tag{3}$$

where L_i are the peptide-site occupation variables: $L_i = 1$ is site i is occupied by a peptide site, otherwise $L_i = 0$. ν is related to the direct lipid-peptide van der Waals-like interaction, which is associated with the interfacial hydrophobic contact of the two molecules, while γ is related to the hydrophobic effect. Since the hydrophobicity of typical α-helix side chains is about half of that of the hydrocarbon chains (Tanford 1973) the value of the mismatch interaction parameter in Eq. (2) is taken to depend on whether the bilayer hydrophobic thickness or the peptide hydrophobic length is the larger.

The interaction between a lipid and the hydrophilic region of a peptide (in the following denoted 'peptide$_\phi$') is formulated in terms of a repulsive *mismatch* interaction whenever $d_P < d_{m,i}$, and in terms of an effective attractive interaction whenever $d_P > d_{m,i}$. The lipid-peptide$_\phi$ Hamiltonian is thus expressed as follows:

$$H^{\phi}_{\mathrm{L-P}} = \Pi \left(\frac{A_P}{7} \right) \sum_i L_{\phi,i} + H^{vdW}_{\mathrm{L-P}} + \begin{cases} \dfrac{(2\gamma)}{2} \displaystyle\sum_{\langle i,j \rangle} \sum_m |d_{m,i} - d_P| L_{m,i} L_{\phi,j}, & \text{if } d_P < d_{m,i} \\ \uparrow \textit{ repulsive } \uparrow & \\ \downarrow \textit{ attractive } \downarrow & \\ -\dfrac{\gamma_w}{2} \displaystyle\sum_{\langle i,j \rangle} \sum_m |d_{m,i} - d_P| L_{m,i} L_{\phi,j}, & \text{if } d_P > d_{m,i} \end{cases} \tag{4}$$

where $L_{\phi,i}$ are peptide$_\phi$-site occupation variables: $L_{\phi,i} = 1$ if the site i is occupied by one of the peptide$_\phi$ sites, otherwise $L_{\phi,i} = 0$. $L_{m,i}$, L_i and $L_{\phi,i}$ satisfy a completeness relation at each lattice site, $(\Sigma_m, L_{m,i}) + L_i + L_{\phi,i} = 1$. The repulsive interaction energy term in Eq. (4) is the same as the repulsive term in Eq. (2). It accounts for the fact that whenever $d_P < d_{m,i}$ it costs energy to have lipid acyl chains exposed to the strong polar environment of water. The addition of a repulsive interaction energy term is instead motivated by the fact that if the ϕ-region of each peptide is exposed to the aqueous environment it prefers to form hydrogen bonds with it, or with a ϕ-region of another peptide, rather than being exposed to the hydrophobic lipid chains (Meyer 1992). This term also contains energy contributions due to van der Waals-like, and electrostatic forces occurring between the ϕ-regions of the peptides. γ_w is associated with the interaction of the ϕ-region of the peptide with the aqueous environment and it can be related qualitatively to the mean hydrophobicity (Engelman et al. 1986) of the ϕ-region of each peptide.

In addition to the lipid-peptide interactions described above, the possible existence of van der Waals-like forces, electrostatic forces, and the formation of hydrogen bonds between peptide polar surfaces (Stickle et al. 1992; Chothia 1976) can induce a direct attractive interaction between the ϕ-regions of the peptides, whose energy is assumed to be proportional to the interfacial contact between the two molecules. The peptide$_\phi$-peptide$_\phi$ Hamiltonian can thus be formulated as follows:

$$H^{\phi}_{\mathrm{P-P}} = -\frac{\gamma_\phi}{2} \sum_{\langle i,j \rangle} d_P L_{\phi,i} L_{\phi,j}. \qquad \uparrow \textit{ attractive } \uparrow \tag{5}$$

The total Hamiltonian of the model may then we written as:

$$H = H_{\mathrm{L-L}} + H_{\mathrm{L-P}} + H^{\phi}_{\mathrm{L-P}} + H^{\phi}_{\mathrm{P-P}}.$$

It can easily be seen that when $\gamma_{\mathrm{w}} = -\gamma$ and $\gamma_{\phi} = 0$ Eqs. (2)–(5) describe the lipid-peptide interaction energy for the case of fully hydrophobic peptides. Therefore, the interaction terms, Eqs. (4) and (5) can be considered as a perturbation term to the Hamiltonian Eqs. (1)–(3), which has already been used in the past to deal with single-site hydrophobic protein (Sperotto and Mouritsen 1991).

Calculational method

The microscopic and the thermodynamic properties of the model have been calculated by standard Metropolis Monte Carlo stimulation techniques (Mouritsen 1990), which give access to microscopic features of the system and hence provide the lateral organization of the membrane components. The thermal equilibrium is achieved by a combination of Glauber and Kawasaki dynamics (Mouritsen 1984). The Monte Carlo method with Glauber dynamics was used for the single-chain conformational excitations regarding the internal transition between two of the ten possible conformational states of the acyl chains. Kawasaki dynamics were used for the lipid and peptide diffusive exchange. The elementary move of a peptide molecule is assumed to correspond to translation of one lattice spacing toward one of the six possible directions on the lattice, and to a simultaneous rotation of sixty degrees. The three acyl chains that have to be displaced in front of a 'moving-rotating' peptide are then translocated to the vacant sites behind the peptide.

In order to assess the state of peptide aggregation on the lattice, the following functions have been calculated from an ensemble of typical equilibrium configurations: $P(n)$, $R(L)$ and $N_{\phi\phi}$, where $P(n)$ is defined as the equilibrium probability of finding a cluster of n peptide monomers, and $R(L)$ is the peptide concentration profile as a function of the distance L from each peptide surface (i. e. at the interface with the lipid chains), where L indexes the lattice site layers around each peptide. Two or more peptides in close contact define a cluster. $P(n)$ is calculated by counting the number of peptide clusters (or peptide monomers if $n = 1$) each containing n peptides, and dividing it by the total number of clusters (and peptide monomers) present in each configuration. $R(L)$ is calculated by counting the number of peptides present in the layer L, and by dividing this number by the total number of molecules (peptides and lipids) present in that layer. $N_{\phi\phi}$ is the equilibrium probability of finding pairs of nearest-neighbour peptide$_{\phi}$ lattice sites belonging to two distinct peptides. $N_{\phi\phi}$ is normalized to the total number of pairs of nearest-neighbour peptide lattice sites which belong to two distinct peptides.

The simulations were performed on a triangular lattice with 40×40 sites. If N is the number of peptides in the lattice and each peptide can occupy seven lattice sites, the corresponding molar concentration, x, is given by $x = N/(1600 - 6N)$. Each system was equilibrated for 20,000 (10,000 for the data in Fig. 2) Monte Carlo steps per site, and statistical averages were calculated over 20,000 (10,000 for the data in Fig. 2) equilibrium configurations, each of which is "separated" by 10 Monte Carlo steps per site. Lattices with 60×60 and 80×80 sites have also been considered in order to estimate finite size effects (Mouritsen, 1990). However it was found that the results do not depend on lattice size in any significant manner.

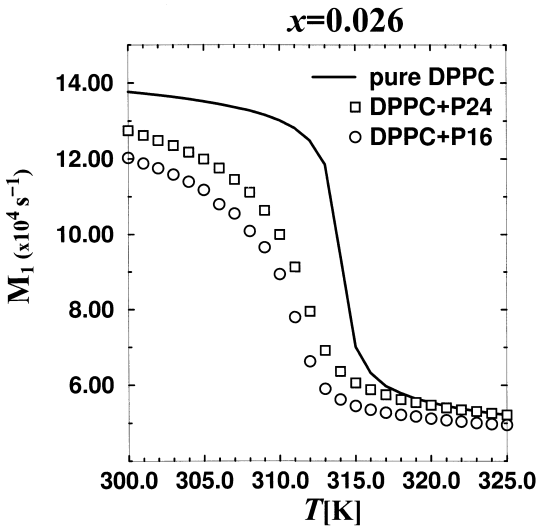

Fig. 2 Calculated 'first moment', M_1, as defined in the text, versus temperature for pure DPPC bilayer (——), and for lipid-peptide mixtures with peptide P 24 (□ □ □), and peptide P 16 (○ ○ ○). The molar concentration of the peptides is: $x = 0.026$

Results

In order to assess the influence of the interaction energy terms Eqs. (2)–(5) on the peptide association, six representative cases (I–VI) are discussed, which differ from one another in the different choices of γ_{ϕ}, γ_{w} and d_{P}.

The values of the parameters γ and ν are the same in all cases: $\gamma = 0.03 \times 10^{-13}$ erg Å^{-1}, $\nu = 0.015 \times 10^{-13}$ erg Å^{-1}. These values were chosen by considering the Hamiltonian energy function for hydrophobic peptides, Eqs. (1)–(3), and by trying to reproduce qualitatively the temperature-composition phase diagram which has been derived from calorimetric and spectroscopic experimental data for the binary mixtures of DPPC and hydrophobic polypeptides made by 24 leucine residues (P_{24}) (Morrow et al. 1985). However, in doing so one should keep in mind the following: On the one hand, it is difficult to obtain accurate experimental phase diagrams, since the experiments do not provide the free energy of the system, but only some derivatives of it, such as specific heat and various spectroscopic order parameters. The positions of phase boundaries are often difficult to determine based on such derivatives. On the other hand, accurate phase boundaries are also

difficult to obtain from standard Monte Carlo simulation techniques in the canonical ensemble (Mouritsen 1990). It should also be pointed out that the interaction parameters γ and ν are obtained by assuming that the peptides are inserted perpendicularly to the lipid bilayer plane, and are not subjected to tilting, even in the case where d_P is much larger than the hydrophobic thickness of the lipid bilayer. Whether long peptides tilt where embedded in the lipid bilayer is still a matter of debate (Nezil and Bloom 1992). Molecular dynamics calculations have shown that the tilting of the hydrophobic peptide of glycophorin requires only an energy of the order of thermal fluctuations (Edholm and Jähnig 1988). A further problem concerns the value of the hydrophobic length of the α-helical peptide P_{24} which forms one of the model inputs. Spectroscopic studies (Zhang et al. 1992) suggest that in the case of P_{24} the standard way to evaluate the peptide hydrophobic length, by multiplying the number of amino acid *per* α-helix turn by the distance between each turn in the helix, overestimates its value (R. N. McElhaney, private communication). Therefore, it was assumed here that the 24-leucine peptide has a hydrophobic length of 30.6 Å, instead of 36 Å. Molecular dynamics studies of simple models of membrane hydrophobic α-helical peptides (Stouch et al. 1994) also tend to indicate that at the lipid/water interface the α-helix can unfold (thus forming hydrogen bonds with water or the lipid head groups), therefore the effective hydrophobic length of the peptide should be shorter than that calculated in the standard way.

With the above values of the interaction parameters γ, ν, and from the microscopic model (Eqs. (1)–(3)) one can make theoretical predictions for the thermodynamic properties of DPPC lipid bilayers with hydrophobic peptides. Figure 2 shows the calculated values of the 'first moment', M_1, versus temperature; M_1 is an order parameter which can be derived experimentally from the quadrupole splittings in the nuclear magnetic resonance spectra of perdeuterated DPPC. The first moment is linearly related to the orientational order of the acyl chains, $\langle S_{CD} \rangle$, (Nezil and Bloom 1992); M_1 was calculated from the following expression (Ipsen et al. 1990 a):

$$M_1 = (2\pi)/(3\sqrt{3})\,\delta\nu_{max}\,\langle S_{CD}\rangle\,, \qquad (6)$$

where $2\delta\nu_{max}$ is the maximum value of the quadrupolar splitting for a deuteron on a CH-bond: $\delta\nu_{max} = 125$ kHz. To a good approximation M_1 is proportional to the mean hydrophobic thickness of the bilayer, $2\langle d_L \rangle$, (Mouritsen 1990): $\langle S_{CD}\rangle = 1.8\,\langle d_L\rangle/d_g - 0.8$, where d_g is the acyl chain length in the all-*trans* configuration, which in the Pink model is set equal to $d_g = 18.75$ Å for DPPC. M_1 was calculated for the following three systems: pure DPPC, DPPC with peptide P_{24} and DPPC with a peptide having a hydrophobic region with sixteen leucines residues, P_{16}. The results shown in Fig. 2 refer to a peptide molar concentration of $x = 0.026$. For the pure system, the dramatic drop in the moment over a narrow temperature interval around T_m signals a phase transition. Below T_m, both peptides tend to decrease the acyl chain order of the lipids, although to a different extent depending on the degree of mismatch between the bilayer and the peptide hydrophobic thickness. Above T_m, the peptides only slightly perturb the pure lipid bilayer order parameter. These data compare well with the available experimental results, which were obtained by Huschilt et al. (1985) using perdeuterated DPPC, as shown in Fig. 3 of their paper. The melting temperature of the pure system used in their experiments occurs a few degrees below T_m owing to the deuteration of DPPC.

γ_w and γ_ϕ are the remaining model parameters. Their values are somehow related to the value of γ; although a quantitative estimate of the values of these parameters is not possible, a qualitative estimate of what is the largest possible value for γ_w relative to the value of γ, could be made by looking at the hydrophobicity scale calculation of the free energy, E, for transferring residues in a α-helix from a non-polar to a polar environment (Popot and de Vitry 1990). For the most hydrophilic amino acid, arginine, the absolute value of E is at least four times larger than that of the hydrophobic residue leucine. Case V shows how the lipid-peptide mixture behaves for the particular choice $\gamma_w = 5\gamma$ (and assuming $\gamma_\phi = \gamma_w$). However, since the aim of the present work was to explore the physical conditions necessary to induce peptide clustering, cases other than case *V* were considered, which differ from one another in the different choices of γ_ϕ, and γ_w. In the following the values of γ_ϕ and γ_w are given in units of the mismatch parameter γ.

In the six cases discussed below, three significant peptide hydrophobic lengths, d_P, have been considered: d_f, d_{f-g}, and d_g, where $d_f = 11.25$ Å, $d_g = 18.75$ Å and $d_{f-g} \equiv (d_f + d_g)/2 = 15$ Å. These values were chosen in order to test different matching conditions between the peptide hydrophobic length and the bilayer hydrophobic thickness. In fact, the value of d_f corresponds to the lipid chain length in the fully melted fluid state, while the value of d_g corresponds to the value of the lipid chain length in the all-*trans* configuration (Pink 1980). For each representative case, the results for the three chosen d_P values are discussed.

The data discussed below are mostly obtained from Monte Carlo simulations performed on a lattice with a peptide molar concentration of $x = 0.026$ corresponding to 37 lipid molecules *per* peptide. However some results will be discussed which refer to a lattice having peptide molar concentrations of $x = 0.01$ and $x = 0.036$, corresponding to 26 and 100 lipids *per* peptide, respectively.

In order to understand the influence of the interaction energy terms, Eqs. (2), (4) and (5), on the lateral organization of the lipid-peptide mixtures, the results from each case studied have been considered in relation to a system with the same peptide concentration, but with a truly random distribution of peptides. This would be the case when the peptide lateral distribution is solely governed by the entropy of mixing, i.e. for a system at an infinitely high temperature.

The results from the simulations refer to a temperature $T = 320$ K, which is a few degrees above the melting temperature, T_m, of the pure DPPC bilayer: 314 K. Below the results are reported in two parts. The first part refers to the

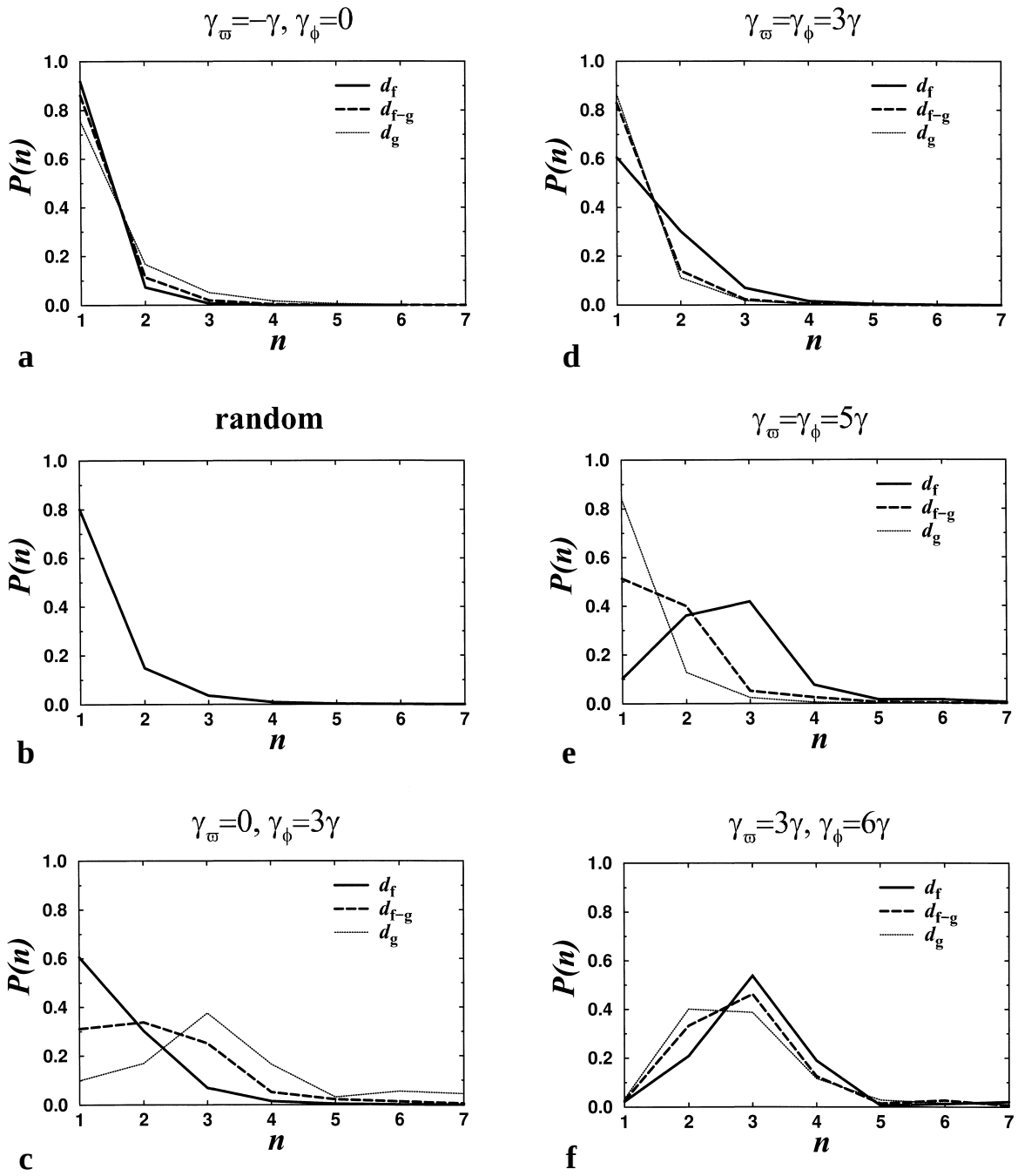

Fig. 3 a–f The equilibrium probability, $P(n)$, of finding a cluster of n peptides (as defined in the test) for three peptide hydrophobic lengths: d_f (——), d_{f-g} (- - -), d_g (——), and for different combinations of the interaction parameters γ_w, γ_ϕ. **a** Hydrophobic peptides ($\gamma_w = -\gamma$, $\gamma_\phi = 0$); **b** Random distribution of peptides; **c** Hydrophilic peptides ($\gamma_w = 0$, $\gamma_\phi = 3\gamma$); **d** Hydrophilic peptides ($\gamma_w = \gamma_\phi = 3\gamma$); **e** Hydrophilic peptides ($\gamma_w = \gamma_\phi = 5\gamma$); **f** Hydrophilic peptides ($\gamma_w = 3\gamma$, $\gamma_\phi = 6\gamma$)

case of fully hydrophobic peptides, while the second part refers to the case of peptides which can have a hydrophilic region in a third of their transbilayer surface at the interface with the bilayer lipid chains.

Hydrophobic peptides

I. $\gamma_w = -\gamma$, $\gamma_\phi = 0$ (Fig. 3 a, b, Fig. 4 a)

When the peptides are completely hydrophobic, the probability, $P(n)$, of finding clusters with n peptide monomers is shown in Fig. 3 a, while Fig. 3 b shows the behaviour of $P(n)$ vs. n for a truly random distribution of peptides. The three curves in Fig. 3 a refer to the systems having peptides with $d_P = d_f$, d_{f-g}, d_g, respectively. When $d_P = d_g$ the *mismatch* interaction (term in Eq. (2)) has only a marginal effect on peptide association. Compared to the random case, the probability of finding dispersed peptides is slightly de-

creased, and consequently the probability of finding peptide dimers and trimers is also increased. The opposite tendency occurs when $d_P = d_f$. For the case of $d_P = d_f$, $R(L)$ has a peak at $L = 3$ instead of at $L = 2$ as in the case of $d_P = d_g$. This implies that the short peptides tend to remain separated from one another and prefer to be intercalated by a layer of lipids. For the three different peptide hydrophobic lengths considered here, $R(L)$ decays very rapidly to a constant value, R_0, which for very large L will approach the value corresponding to the global molar concentration of the lipid-peptide mixture: 0.026 (in the random case $R(L) = R_0$ whenever $L \geq 2$). These results are a consequence of the fact that in the fluid phase the lipid-lipid attractive van der Waals-like interaction energy Eq. (1) is much weaker than the lipid-peptide one (Eq. (3)) for all of the three considered values of d_P, thus forcing the peptides to be mono-dispersed. However when $d_P = d_g$ the repulsive mismatch interaction (Eq. (2)) will tend to force the peptides to cluster to reduce the free energy cost of having nonpolar moieties exposed to water, thus counteracting the van der Waals-like interaction and tending to restore the random distribution.

Figure 4 a shows a snap-shot of a typical micro configuration for the case of $d_P = d_g$. Although this mixture is in the fluid phase, a number of small gel-like lipid domains appear in the vicinity of the peptides. This is due to the fact that: 1) for the temperature in question, the pure lipid bilayer is still characterized by density fluctuations (Ipsen et al. 1990 b), and therefore a number of gel-like lipids are still present in the fluid phase; 2) the mismatch interaction energy term in Eq. (2) effectively favours gel-like chains in the vicinity of each peptide in order to shield its hydrophobic surface from the aqueous environment. In fact, a comparison between the calculated values of the average hydrophobic thickness of the pure DPPC lipid bilayer and the lipid bilayer containing the peptides ($d_P = d_g$) indicates that the presence of these long peptides induces a slight increase (1.3 Å) of the average hydrophobic thickness of the lipid bilayer with peptides, compared to the pure one.

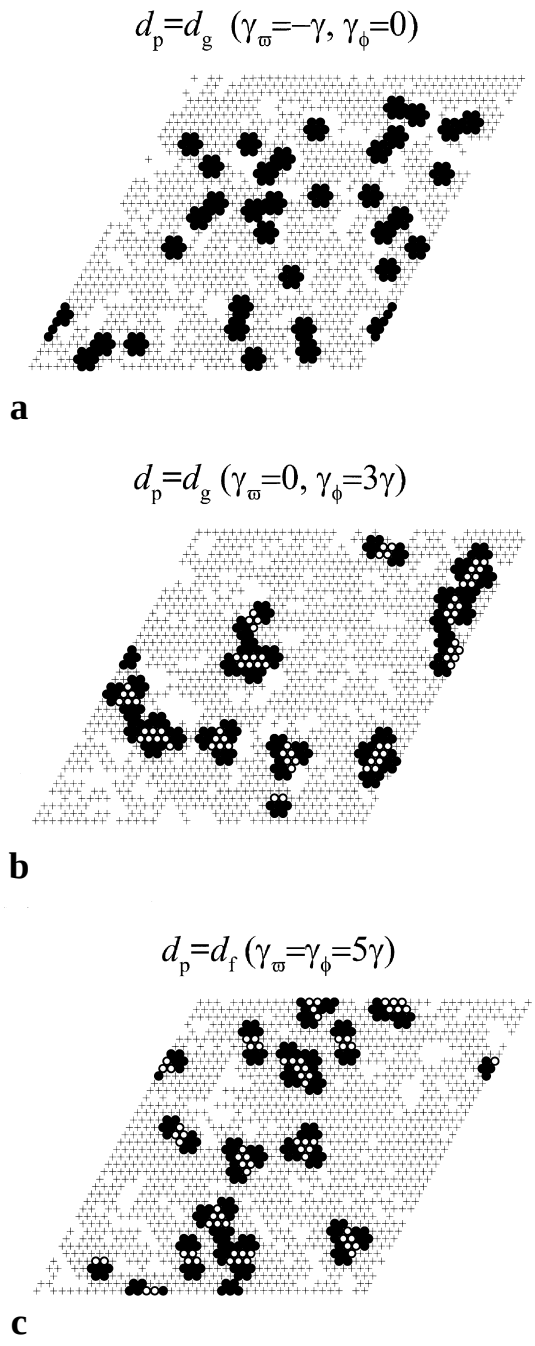

Fig. 4 a–c Snapshot of a typical microconfiguration of the lattice for a DPPC-peptide mixture in the fluid phase, for different values of the peptide hydrophobic length, d_P, and the interaction parameters γ_w, γ_ϕ. **a** Hydrophobic peptides with $d_P = d_g$ ($\gamma_w = -\gamma$, $\gamma_\phi = 0$); **b** Hydrophilic peptides with $d_P = d_g$ ($\gamma_w = 0$, $\gamma_\phi = 3\gamma$); **c** Hydrophilic peptides with $d_P = d_f$ ($\gamma_w = \gamma_\phi = 5\gamma$). The symbols are as follows: ● = peptide segment (hydrophobic), ○ = peptide segment (hydrophilic), + = fluid DPPC acyl chain, no symbol = gel DPPC acyl chain

Partially hydrophilic peptides

II. $\gamma_w = 3\gamma$, $\gamma_\phi = 0$

In the case when there is no attractive interaction between the peptide polar sides ($\gamma_\phi = 0$), increasing γ_w from a negative (hydrophobic case) to positive values, $\gamma_w = \gamma$, $\gamma_w = 2\gamma$, $\gamma_w = 3\gamma$, has no substantial effect on peptide assembly, no matter what d_P is. Most of the peptides are in the monomeric state. For all of the three chosen values of d_P, the probability of finding peptide dimers is approximately the same as discussed in *I* for the case when $d_P = d_f$. When $d_P > d_f$, the effect of the lipid-peptide$_\phi$ attractive interaction energy term in Eq. (4) is actually to reduce the influence of the mismatch interaction energy term in Eq. (2). Therefore peptides with $d_P = d_g$ will remain slightly more polydispersed than in the random case. Furthermore, in the case when $d_P > d_f$, the results from the simulations show that the average chain length of the lipid chains that are nearest neighbours to each peptide is approximately 1 Å shorter than what is found in the corresponding case discussed in *I*. This is a consequence of the fact that the attractive interaction energy term in Eq. (4) tends to favour

fluid chains in the vicinity of the polar region of each peptide, while the mismatch interaction term in Eq. (2) favours chains with gel-like states in the vicinity of non-polar peptide regions, since these match better the peptide hydrophobic lengths, and are thus energetically favourable.

III. $\gamma_w = 0$, $\gamma_\phi = 3\gamma$ (Fig. 3 c, Fig. 4 b)

A direct interaction between the ϕ-regions of the peptides is now operative. Such interaction has a pronounced effect on peptide association, as seen in Fig. 3 c, which shows the behaviour of $P(n)$ vs. n. The longer the peptides are the more the tendency to cluster is pronounced, since the attractive peptide$_\phi$-peptide$_\phi$ interaction energy term Eq. (5) is proportional to d_P, and is thus higher for higher d_P. In the case of $d_P = d_f$ the probability of finding peptide clusters decreases monotonically with n. In the case of $d_P = d_g$, $P(n)$ has a maxium for $n = 3$, but still remains high for $n = 4$. Simulations done on mixtures with peptides with $d_P = d_g$ and peptide molar concentrations $x = 0.01$ and 0.036 give similar results as in this latter case.

Figure 4 b shows a typical lattice configuration when $d_P = d_g$. Helices seems to assemble in such a way as to turn their hydrophilic region away from the lipid chains. This is confirmed by the calculated values for the function $N_{\phi\phi}$. For a truly random distribution of amphiphilic peptides with a molar concentration such as the one chosen here, one finds that $N_{\phi\phi} = 0.11$, while for the case in question (as well as for the other cases when $d_P = d_f$ and d_{f-g}) $N_{\phi\phi}$ is at least six times higher than in the random case. Therefore, independent of the peptide hydrophobic length, the peptides in the clusters arrange themself in such a way as to shield their ϕ-region from the strong apolar environment of the lipid chains and, on average, they tend to form trimers and tetramers. Furthermore, the snap-shot in Fig. 4 b shows that these aggregates tend to cluster in even larger units. This is confirmed by the fact that when $d_P = d_g$ $R(L)$ has a high peak for $L = 2$, and $P(n)$ still has a significant value at $n = 7$.

IV. $\gamma_w = \gamma_\phi = 3\gamma$ (Fig. 3 d)

The results from the simulations in the case when γ_w, γ_ϕ are equal but less then 3γ, show that $P(n)$ does not differ from that of a random mixture (see Fig. 3 b) in any significant manner, independent of the choice of d_P. However, in the case of peptides with $d_P = d_f$, when γ_w, γ_ϕ are equal to, or greater than, 3γ, the probability of forming peptide dimers is approximately double that in the random case, as illustrated in Fig. 3 d; while, for peptides with $d_P = d_g$, d_{f-g}, $P(n)$ does not differ substantially from that of a random mixture (see Fig. 3 b). However, the values of $N_{\phi\phi}$ depend on d_P, although they are always higher than in the random case. When $d_P = d_f$, $N_{\phi\phi}$ is approximately seven times higher than in the random case, and decreases for the case of peptides with larger d_P. Although for the case of peptides with $d_P = d_g$, d_{f-g} the values of the functions $P(n)$ and $R(L)$ are close to the ones of a random distribution of peptides, whenever peptide clusters (dimers mostly) are formed, the interaction strengths' considered in this case are such as to affect the orientation of the peptides in the clusters, so that the peptides have the tendency to shield their hydrophilic sides from the lipid environment.

V. $\gamma_w = \gamma_\phi = 5\gamma$ (Fig. 3 e, Fig. 4 c)

A further increase of γ_w and γ_ϕ increases substantially the probability of finding peptide aggregates, at least for peptides with $d_P = d_f$, d_{f-g}. The behaviour of the function $P(n)$ is shown in Fig. 3 e. In contrast, when $d_P = d_g$ the peptides have a slight tendency to remain 'separated' from one another by a layer of lipid chains (cf. Fig. 3 c), as indicated by the behaviour of $R(L)$ vs. L: When $d_P = d_f$, d_{f-g}, $R(L)$ has a peak at $L = 2$, while, when $d_P = d_g$, $R(L)$ shows a less pronounced peak at $L = 3$.

In the case of peptides with $d_P = d_f$, the snap-shot of a typical lattice configuration is shown in Fig. 4 c. It can be seen that the peptides tend to aggregate by keeping their hydrophilic region in contact with the hydrophilic region of other peptides. This observation is confirmed by the calculated values of $N_{\phi\phi}$, which (for all three values considered for d_P) are at least four times larger than that characteristic of a random distribution of peptides.

VI. $\gamma_w = 3\gamma$, $\gamma_\phi = 6\gamma$ (Fig. 3 f)

The results (not shown) from the simulations indicate that, in general, no matter what the chosen d_P is, peptide aggregation occurs when the interaction parameters are such that $\gamma_\phi > \gamma_w > 0$. The behaviour of $P(n)$ vs. n is shown in Fig. 3 f: For the three chosen d_P, $P(n)$ is very small for $n = 1$, has a peak at $n = 2$ (or 3) and then decays toward zero for n greater than four; in contrast, when γ_ϕ is reduced by a factor two (as considered in case *IV*), the probability of finding dispersed peptides is higher than the probability of finding aggregates with two or more peptides, as can be seen in Fig. 3 d. For the three chosen peptide hydrophobic lengths, the values of $N_{\phi\phi}$ indicate that the peptides in the aggregates orient in such a way to shield their hydrophilic region from the acyl chain hydrophobic environment.

In summary, the results discussed in cases *II*–*VI* show the following trend: when $\gamma_w = \gamma_\phi > 2\gamma$, peptide oligomers form in the fluid phase only for $d_P = d_f$, while peptides with $d_P = d_g$ do not cluster. Although the direct peptide$_\phi$-peptide$_\phi$ interaction is stronger for $d_P = d_g$ than for the case of $d_P = d_f$, the interaction energy term related to γ_w dominates the term related to γ_ϕ. If $\gamma_w = 0$ and $\gamma_\phi > 0$, peptide assembly occurs both for $d_P = d_g$ and d_f; However it is more pronounced when $d_P = d_g$, owing to the attractive peptide$_\phi$-peptide$_\phi$ interaction which is proportional to the peptide hydrophobic length.

Discussion

A model has been used to analyse the conditions under which the state of aggregation of transbilayer peptides is lipid-mediated, and to what extent it may be induced by direct interactions between the hydrophilic surfaces of the peptides.

In the case of *hydrophobic* peptides, the results from the simulations show that the existence of a mismatch between the peptide hydrophobic length and the lipid hydrophobic thickness has only a marginal effect on the peptide aggregation. Similar results were obtained from previous model studies of lipid-protein interactions for single-site proteins (Sperotto and Mouritsen 1991). The hydrophobic mismatch seems to be relevant for determining the phase behaviour of lipid-peptide mixtures rather than for determining the state of association of peptides in lipid bilayers, as already shown by calorimetric and nuclear magnetic resonance studies of the interaction of hydrophobic α-helices with phospholipid bilayers (Zhang et al. 1992; Morrow et al. 1985), as well as by theoretical studies (Sperotto and Mouritsen 1991; Zhang et al. 1993). For peptide-lipid mixtures which are in one macroscopic phase, fluid or gel, the lipid-mediated peptide lateral dispersion is more controlled by factors such as the interplay between the strength of the lipid-lipid and lipid-peptide direct interactions, as well as the temperature, rather than the hydrophobic mismatch.

In the case of *amphiphilic* peptides, the results from the model study indicate that direct peptide$_\phi$-peptide$_\phi$ interactions are necessary to induce peptide association in the fluid phase. No matter how strong the attractive interaction is between the hydrophilic region of the transmembrane peptides and the polar environment, peptide association into clusters does not occur unless the direct peptide$_\phi$-peptide$_\phi$ interactions are present. In this case, peptides within each cluster are oriented in such a way as to shield their ϕ-regions from the hydrophobic environment. Furthermore, the average number of peptides forming the clusters depends on the degree of mismatch between the peptide hydrophobic length and the bilayer hydrophobic thickness: the lower the mismatch is the higher this number is (see, for example, Fig. 3e). This result is consistent with the results from neutron in-plane scattering experiments which were used to find the mean number, $\overline{n}$, of alamethicin monomers present in the aggregates forming aqueous pores in dilauroyl phosphatidylcholine (DLPC) and diphytanoil phosphatidylcholine (DPhPC) bilayers in the fluid phase (He et al. 1995). The results from the neutron scattering experiments show that, although the mismatch between the fluid lipid bilayer and peptide hydrophobic thickness is higher for alamethicin in DLPC than in DPhPC, $\overline{n}$ is smaller in DLPC ($\overline{n} = 8$) than in DPhPC ($\overline{n} = 11$).

It is worth adding that, although the interpretation of the theoretical data given above is mainly based upon qualitative considerations regarding interaction energy terms of the model Hamiltonian, the results presented in this paper are obtained from a simulation technique which correctly deals with the entropy of mixing of the system. Furthermore, although the results refers only to one temperature, they can provide a guideline for the characterization of lipid-peptide mixtures in the fluid phase.

In the model formulation the interactions between the peptide ϕ-regions with other peptide non-polar regions are neglected, although hydrogen bonds are also formed between those peptide regions (Stickle et al. 1992). The modelling of possible long range forces acting between the molecules is not considered here, since only nearest-neighbour site interactions are dealt with in the lattice formulation of the model. Furthermore, the calculations did not focus on the 'kinetics' (life-time and stability) of the peptide aggregates but only on the physical conditions necessary for their existence. In addition, the microscopic behaviour of the peptide-lipid mixtures considered here shows that the largest peptide clusters are tetramers; this is due to constraints imposed by the geometry of the lattice (since the peptide hydrophilic area was defined to be one third of the total peptide surface in contact with the lipid chains (see Fig. 1) and by the fact that only one type of peptide is considered here.

The model presented in this paper is admittedly still crude and some of the interactions, and motional modes, present in biomembranes are still missing; However, at this stage of the theoretical modelling, it is important to understand what are the effects of non-specific physical interactions on the cooperative behaviour of model membranes before one moves on to more realistic and complicated models involving, for example, detailed molecular description of the molecules and their interactions. The work presented here should thus be seen as an attempt in this direction.

By the use of de novo design of model peptides (De Grado et al. 1989) it is possible to define specific model systems. By varying systematically the degree of hydrophilicity/hydrophobicity of the transmembrane peptides, their hydrophobic length, and their concentration in the lipid matrix, one can better define the forces responsible for the insertion of peptides into lipid bilayers and their self-association into aggregates like bundle-proteins; attempts in this direction have already been made (Kiyota et al. 1996; and references therein).

The predictions which arise from model studies such as the one presented here may thus provide a framework for the interpretation of experimental data on model systems, and may also serve as a guideline for future experiments on model membranes.

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