Chapter 27

Interaction of a Model Peptide with a Water–Bilayer System

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We discuss a molecular dynamics study of the alanine dipeptide at the interface between water and a glycerol-1-monooleate (GMO) bilayer. The dipeptide is interfacially active and incorporates into the bilayer without disrupting its structure. The interfacial region that is readily penetrated by the dipeptide spans the entire head group portion of the bilayer. The polar groups of the alanine dipeptide mostly remain well solvated by water and the oxygen atoms of GMO, and conformations of the dipeptide are characterized by (ϕ,ψ) angles typical of α-helix and β-sheet structures. When the molecule is deeper in the bilayer, the C_{7eq} state also becomes stable. The barrier to the isomerization reaction at the interface is lower than in bulk phases. After 7 ns of trajectories, the system is not fully equilibrated, due to slow collective motions involving GMO head groups. These result in decreased mobility and lower rates of isomerization of the dipeptide at the interface.

Peptides with affinity for membrane surfaces exhibit a broad range of cellular activities. They are hormones, antibiotics, toxins, and fusogenic and signal peptides (*1-3*). Their biological functions are determined by their secondary structure at the membrane surface. This secondary structure is often different at the membrane–water surface than in aqueous solution. Recent studies of the conformational changes induced by the membrane surface in synthetic, model molecules revealed two remarkable features of peptide–membrane interactions. First, short peptides, which are disordered in water, can acquire secondary structure at the water–membrane interface. Second, polarities rather than the specific identities of residues determine the secondary structure of the peptide at membrane surfaces. Peptides with alternating polar/nonpolar residues form β-sheets (*4,5*), while α-helices have polar and nonpolar amino acids arranged so that they match the period of this structure (*5-7*). This indicates that the amphiphilic nature of

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the water–membrane interface, where polar and nonpolar phases exist in direct proximity, affects the conformational preferences of peptides.

Since experimental methods for studying membrane–bound peptides suffer from several difficulties, such as limited residence times of these peptides at the interface, poor water solubilities of some amino acid sequences, and inability to obtain crystal structures in most cases of interest, molecular-level computer simulations can potentially play a significant role in determining how peptides interact with water–membrane interfaces and how these interactions depend on amino acid sequence. Despite the obvious biological importance of these problems, no such simulations have been reported so far, probably due to difficulties in proper treatment of highly anisotropic interfacial environments. However, recent progress in our understanding of the structure and properties of aqueous interfaces (*8*), and water–membrane systems in particular (*9-14*) render realistic simulations of small peptides at membrane surfaces possible.

In this paper, we present results of molecular dynamics simulations of the alanine dipeptide, which is probably the simplest model peptide, at the interface between water and a lipid membrane formed by glycerol-1-monooleate (GMO). This work builds on our previous studies of the water–GMO membrane system (*14*) and the alanine dipeptide at the water–hexane interface (*15*). While this peptide is obviously too short to form a secondary structure, it has been considered a good model for examining conformational preferences of the protein backbone. Several computational studies have show that these preferences depend on the environment. The conformations of the alanine dipeptide corresponding to the local energy minima in the gas phase (*16,17*) and in hexane (*15*) are stabilized by intramolecular hydrogen bonds. In water, the hydrogen bonds are destabilized by hydration effects and the most stable conformations are characterized by ϕ and ψ angles typical of α-helices and β-sheets (*17-19*). At the water–hexane interface both types of conformations are populated (*15*).

Based on our results for the alanine dipeptide in the water–hexane system, we expect that this molecule exhibits interfacial activity at the water–membrane interface. One of our objectives is to determine how this complex interface influences the conformational preferences of the dipeptide backbone. Further, we want to obtain a microscopic description of the environment surrounding the dipeptide and its orientational preferences at the interface. Finally we would like to gain information about the relative time scales of processes affecting the peptide, such as diffusion and conformational transition of the solute, and relaxation of the solvent. These issues are addressed in the Results and Discussion section which follows the description of the system and the methods used.

Method

The system under study consisted of two alanine dipeptide molecules, 72 GMO molecules, and 2274 water molecules in the simulation box of 36.94 Å × 36.94 Å × 150 Å. The chemical structures of the dipeptide and the GMO molecule with the numbering system used in the text are shown in Figure 1a. The head group of GMO contains atoms 18–27; the rest of the molecule constitutes the hydrocarbon tail (atoms 1–17). The GMO molecules were arranged as a planar bilayer perpendicular to the z-direction of the simulation box. The xy-dimensions of the

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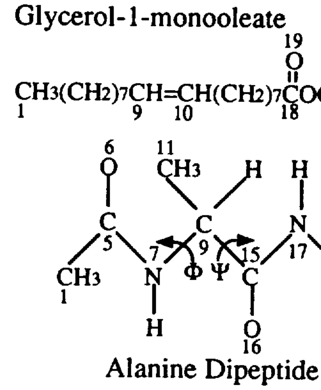

Figure 1. (a) Chemical stru
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α_R (-60,-40), β (-60,150), C_5
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system were set to reproduce the experimental surface density of molecules in the bilayer (*20*). The membrane was located between two water lamellae, each containing 1137 water molecules, as is schematically represented in Figure 1b.

The arrangement of the system was the same as that used in our molecular dynamics simulations of the water–GMO bilayer (*14*). In those calculations, it was found that the width of the membrane, measured as the distance between peaks in the density of the head-group atoms on both sides of the bilayer, is 30 Å. This density decreased to 10% of its maximum value at ±19.8 Å from the middle of the bilayer. The width of each water lamella was 26 Å, which correspond to approximately 8 layers of water. On the bilayer side, the water density decreased to 90% and 50% of the value in bulk liquid at ±19.0 and ±16.5 Å, respectively.

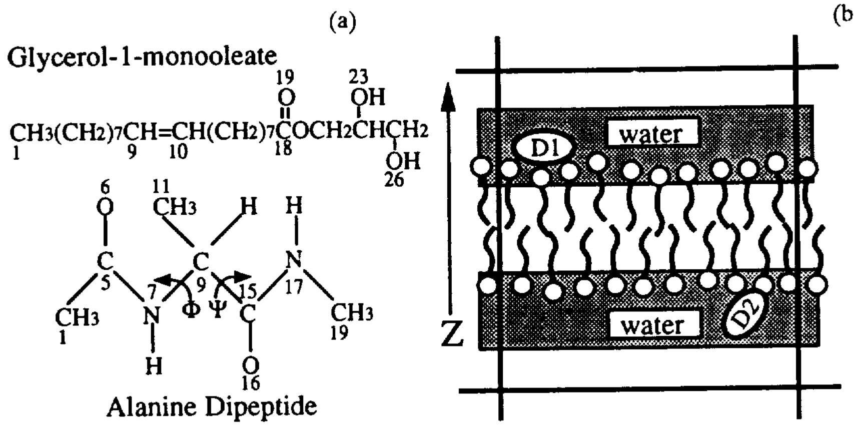

Figure 1. (a) Chemical structures of GMO and the alanine dipeptide. The dipeptide angles ϕ and ψ are marked; (b) Schematic of the water–bilayer system. The alanine dipeptide molecules are shown in the interfacial region.

These results guided our choice for the initial locations of the two alanine dipeptide molecules in the system. They were placed in aqueous solution close to the interface with the bilayer, such that their α carbon atoms (C(9)) were at $z = \pm$ 24 Å from the middle of the bilayer. The locations of the dipeptide molecules in the system is schematically depicted in Figure 1b. Three initial configurations were constructed by placing the alanine dipeptides in configurations of the water–bilayer system drawn from the equilibrium distribution obtained in our previous work (*14*) and removing any water molecules which overlapped the solutes. The x, y-coordinates of C(9) and the molecular orientation of the peptide were chosen at random. The initial conformations of the alanine dipeptide corresponded to the energy minima found for this molecule at the water–hexane interface (*15*)—α_R (-60,-40), β (-60,150), C_5 (-160,150) and C_{7eq} (-60,60), where the ϕ and ψ angles for each conformation are given in parentheses. Other conformations which are also stable at the water–hexane interface, but are separated from this set of conformations by fairly high barriers, such as α_L (60,60) and C_{7ax} (60,-60), were not considered in this work.

Potential Energy Functions. The potential energy functions for GMO and water molecules were the same as those used for the water–bilayer system (*14*). Water molecules were described by the TIP4P model (*21*). In the GMO molecule, all CH_n groups were represented as united atoms, whereby the hydrogen atoms were not explicitly considered and the carbon atom was assigned the mass of the whole group. All bond lengths and bond angles were kept rigid. In this approximation, the potential energy was expressed as a sum of torsional potentials of GMO molecules, van der Waals energies between atoms (or groups) and Coulomb energies between partial charges on water molecules and GMO head groups. The details of the potential energy functions, the atomic partial charges and the van der Waals and torsional parameters were given previously (*14*).

The alanine dipeptide was represented by a flexible model with full atomic detail. The potential energy function contained contributions from bond stretching and bending of planar angles in addition to torsional, van der Waals and Coulomb terms. This potential has been described in our study of the dipeptide at the water–hexane interface (*15*). The relative free energies of stable conformations of the alanine dipeptide in the gas phase and in water, calculated in that work, are in close agreement with the free energies recently obtained by Tobias and Brooks (*17*). The dipeptide–water and dipeptide–GMO potential energy functions were evaluated from the Lorentz–Bertholet combination rules (*22*).

Molecular Dynamics. Three molecular dynamics trajectories, each 1 ns long, were generated, starting from the configurations described above. Assuming that interactions between the solute molecules across the bilayer can be neglected, six independent trajectories for the alanine dipeptide were obtained.

The equations of motion were integrated using the Verlet algorithm with a time step of 2.5 fs. The temperature of the system was 300 K. Minimum image periodic boundary conditions were applied in all three dimensions. The bond lengths and planar angles of the GMO and water molecules were kept fixed by using the SHAKE algorithm (*23*). No constraints were applied to the degrees of freedom of the dipeptide. The intermolecular interactions were truncated smoothly between distances of 7.5 and 8.0 Å, as measured between centers of charge–neutral groups (*14*).

Free Energy Calculations. The stable conformations of the alanine dipeptide in the ϕ, ψ range studied here, α_R, β and C_{7eq} are characterized by approximately the same values of ϕ. Thus, the free energy profiles for transitions between these conformations can be described as a function of only ψ. These profiles, $A(\psi)$, were obtained from the probability distributions, $P(\psi)$, of finding the molecule in a conformation with by angle ψ:

$$A(\psi) = -kTlnP(\psi) \qquad (1)$$

where k is the Boltzmann constant and T is temperature.

As described in the next section, a sufficient number of transitions was observed in some cases to permit a direct calculation of $P(\psi)$ from the molecular dynamics trajectory (after equilibration). In other instances, additional molecular dynamics calculations were needed in which ψ was restricted to different, but

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Results and Discussion

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In Figure 2b we present the
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overlapping ranges. Four such additional trajectories, 0.35 ns long, were obtained in each instance. In these trajectories the angle ϕ remained unconstrained. To ensure uniform sampling of each region and, therefore, improve statistical precision of the resulting $P(\psi)$ an additional external potential $U_{ext}(\psi)$ was sometimes added. Then, $A(\psi)$ in a given range of ψ is obtained as:

$$A(\psi) = -kTlnP(\psi) - U_{ext}(\psi) \tag{2}$$

and the full free energy profile is constructed by requiring that $A(\psi)$ be a continuous function of ψ (*24*).

Results and Discussion

Interfacial Activity of the Alanine Dipeptide. The time evolution of the positions of the six alanine dipeptide molecules along the direction perpendicular to the interface is shown in Figure 2a. These positions are defined as the absolute values of the z-coordinate of atom C(9) measured from the mid–plane of the bilayer. The dipeptide molecules move from water to the interface in the first 0.3–0.5 ns of the molecular dynamics trajectories. Each then explores a wide interfacial region occupied by GMO head groups and water molecules, which extends approximately from $|z|$=10 Å to $|z|$=21 Å. There are, however, differences in the degree to which the molecules penetrate the head group portion of the bilayer. In particular, molecule 6 appears to be located, on average, deeper in the bilayer than other molecules. As will be shown further in the paper, this molecule behaves somewhat differently than molecules 1-5 and, therefore, will be discussed separately. A question may be raised whether molecule 6 has reached its equilibrium position or, alternatively, will continue to move deeper into the bilayer. This issue was resolved by performing an additional 1.3 ns of molecular dynamics trajectory for the system containing this molecule. No deeper penetration of the solute into the membrane was observed during this trajectory.

In Figure 2b we present the free energy profile of the alanine dipeptide in the interfacial region estimated from the probability distribution along z averaged for molecules 1-5 and, separately, from the distribution for molecule 6. We note three important features of these results: First, the alanine dipeptide is interfacially active. This conclusion is further supported by a series of short molecular dynamics trajectories in which the dipeptide was forced into the hydrocarbon core of the bilayer. When the external force was removed and some equilibration was performed, the dipeptide returned to the interface in less than 0.05 ns. A similar interfacial activity is exhibited by the dipeptide at the water–hexane interface (*15*). It was found that this molecule is stabilized at the interface, relative to aqueous and hexane solutions, by 5.5 and 10.0 kcal/mol, respectively. Thus, it appears that, in general, the alanine dipeptide binds to interfaces between water and nonpolar phases.

Second, the interfacial region that is readily accessible to the dipeptide is remarkably broad. The averaged free energy for molecules 1-5 is almost constant in the range 13 < $|z|$ < 20 Å. The results for molecule 6 show that penetration into the bilayer by another 2 Å is also probable. This is different from the situation at the water–hexane interface, where the free energy along z exhibits a much sharper minimum near the plane where the densities of water and hexane decrease to the half of their bulk values. The observed width of the free energy profile at the water–membrane interface does not appear to be unique to the alanine dipeptide. A very similar profile was found for the anesthetically active molecule, 1,1,2-trifluoroethane (*25*). These results suggest that small, neutral solutes active at interfaces between water and nonpolar media experience considerably greater freedom of movement at the water–membrane interface than at the water–oil interface.

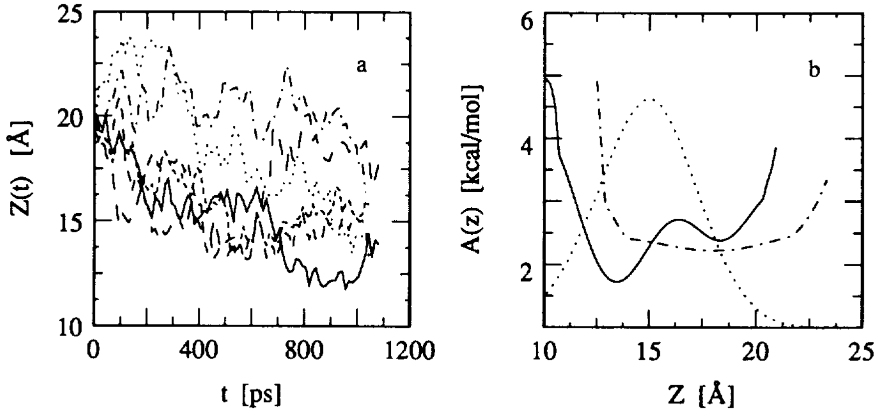

Figure 2. (a) Trajectories of the dipeptide molecules perpendicular to the membrane surface. Run 6 is the solid line; (b) Free energy profiles of the alanine dipeptide as a function of z, for runs 1–5 (dash–dotted line) and run 6 (solid line). The density profile of the GMO head groups (dotted lines) is shown as a reference. The center of the bilayer is located at $z = 0$.

Third, we note that differences between the free energy profiles along z for molecules 1–5 and molecule 6, which did not disappear during the course of the simulations, probably indicate the existence of slow relaxation modes in the solvent which were not fully equilibrated. Other consequences of this effect and structural differences in the environment around molecules 1–5 and molecule 6 will be discussed farther in the paper.

Conformations of the Alanine Dipeptide at the Membrane–water Interface. The trajectories of the six alanine dipeptide molecules yield somewhat different results regarding conformational equilibria and transitions. Molecules 1-3 remained in their initial (after equilibration) α_R or β conformations during the entire trajectory. In contrast, molecules 4–6 underwent isomerization. For molecules 5 and 6, several transition between different conformations were observed, so that it was possible to estimate the free energy profile for the isomerization reaction from the probability distribution of finding different values of ψ during the trajectory. For molecules 3 and 4 this profile was obtained from a series of calculations in which ψ was constrained in different ranges, as described

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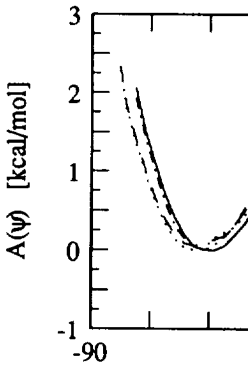

Figure 3. Free energy profi
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in the method section. The results are summarized in Table I and the four free energy profiles are shown in Figure 3.

The observed differences between the trajectories indicate that the system does not fully equilibrate in the course of the simulations. Nevertheless, several conclusions can be drawn from the results. The conformations α_R and β, which are stable in aqueous solution, are also stable at the water-membrane interface. The free energy difference between α_R and β does not change significantly upon transferring the dipeptide from water to the interfacial region. At the interface, this difference ranges from 0.0 to 1.0 kcal/mol for molecules 3–5, compared to 0.4 kcal/mol in the aqueous solution (*15*). The position of the free energy barrier separating the two stable conformations is also similar to that for the dipeptide in water. However, the height of the barrier at the interface is 1.6 kcal/mol (measured from the α_R state), only about half of that in aqueous solution. A statistically significant difference in the relative stabilities of the α_R and β conformations between molecule 3 and molecules 4 and 5 is a consequence of slowly relaxing modes in the system. This point is further underscored by the fact that molecule 6 exhibits a different free energy profile. This profile resembles that obtained previously for the alanine dipeptide at the water–hexane interface (*15*). In addition to the α_R and β conformations, C_{7eq} is also stable. This corresponds to a free energy minimum in hexane but not in water. The barriers are again markedly lower than those in bulk aqueous and hexane phases.

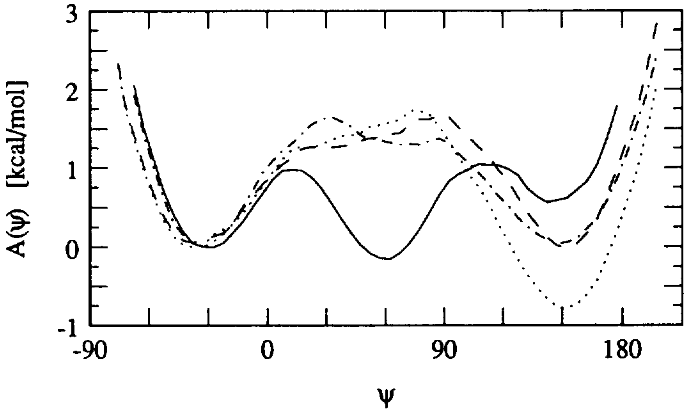

Figure 3. Free energy profiles for $\alpha_R \rightleftharpoons \beta$ isomerization at the membrane interface for molecules 3 (dashed line), 4 (dotted line), 5 (dash–dotted line) and 6 (solid line).

The observed differences in the calculated free energy profiles raise an important question whether an internal solute degree of freedom (angle ψ) is a proper choice for the reaction coordinate. In general, such a choice is satisfactory when other degrees of freedom of the system, in particular those associated with the solvent, equilibrate rapidly, compared to the relaxation time of the reaction. Then, the reaction can be considered as proceeding along the reaction coordinate in the potential of mean force exerted by other degrees of freedom. However, if some of these degrees of freedom relax slowly the rate of the reaction depends on their specific values. This is apparently the case here and, therefore, the calculated free energy profiles depend not only on ψ but also on some "hidden variable(s)"which describe the slow relaxation of the system and can facilitate or, alternatively, impede the isomerization reaction. Examples of such structural "variables" would be water molecules protruding unusually deep into the bilayer to hydrate the solute, or GMO molecules tightly bound to the dipeptide. Such a phenomenon was observed by Benjamin (*26*) in his study of the transfer of an ion from 1,2-dichloroethane to water. He found that the reaction proceeds readily only on fairly rare occasions when a chain of water molecules (a "water finger") develops between the aqueous phase and the ion. Despite extensive analysis, we were unable to identify any such specific interactions in the present case. This points to the participation of collective modes in the slow relaxation of the system. This conclusions is reinforced by the analysis of the solvation of the dipeptide, the structure of the membrane around this molecule, and dynamic properties of the system, presented below.

Table I. Summary of the Results for Conformational Equilibria of the Alanine Dipeptides at the Water–Bilayer Interface

	molecules:					
	1	2	3	4	5	6
conformations[a]	α_R	β	β	α_R,β	α_R,β	C_{7eq},α_R,β
$\Delta A(\alpha_R - \beta)$ [b]	[d]	[d]	-0.8	0	0	0.5 (-0.1)[e]
$\Delta A^{\ddagger}$ [c]	[d]	[d]	1.7	1.6	1.6	1.2

[a] conformations observed in the trajectories with no constraints on ψ; [b] the free energy difference between α_R and β conformations, in kcal/mol.; [c] activation free energy for the $\alpha_R \rightleftharpoons \beta$ isomerization reaction; [d] not calculated; [e] the free energy difference between α_R and C_{7eq} in parenthesis.

Hydration of Alanine Dipeptide at the Interface. Earlier studies of the alanine dipeptide in aqueous solution (*17-19,27*) stressed that the α_R and β conformations are strongly stabilized by water relative to the conformations containing intramolecular hydrogen bonds. Since the same conformations are also stable at the water–bilayer interface, it raises a question to what extent the alanine dipeptide remains solvated at the surface of the membrane. To answer this question we compared the calculated radial distribution functions between oxygen atoms of water and oxygen atoms, methyl groups and nitrogen atoms of the dipeptide in aqueous solution and at the water–bilayer interface. The distribution functions for the water oxygen atoms and the oxygen atoms of the dipeptide are shown in Figure 4a. From the average of runs 1–5, it is clear that the peptide becomes partially dehydrated upon transfer from bulk water to the interface. Integration of the oxygen–oxygen radial distribution functions

to the first minimum yields
dipeptide in aqueous solution
similar pattern of dehydratio
nitrogen atoms (not shown).

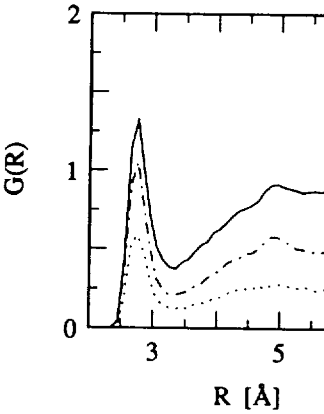

Figure 4. (a) Radial distr
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carbon atoms.

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yields a solvation number of
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The radial distribution fu
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the water–hexane interface. As

to the first minimum yields hydration numbers of 2.2 and 1.6 for the alanine dipeptide in aqueous solution and at the water-bilayer interface, respectively. A similar pattern of dehydration is observed for the methyl groups (Figure 4b) and nitrogen atoms (not shown).

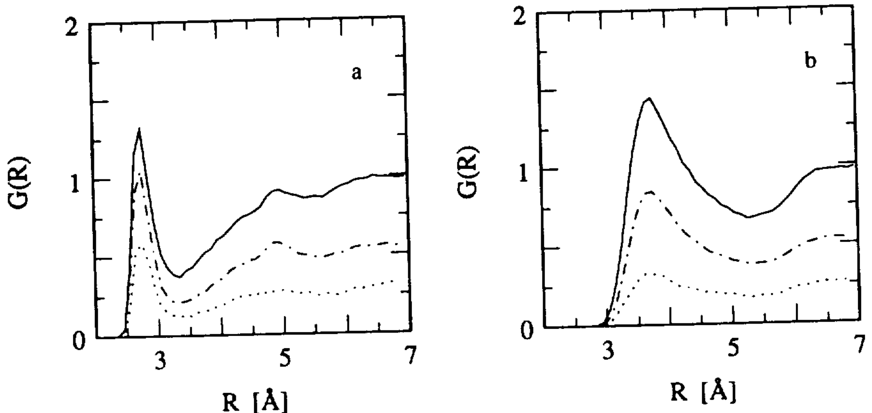

Figure 4. (a) Radial distribution function between the water oxygen and the alanine dipeptide oxygen atoms in bulk water (solid line), averaged over molecules 1-5 (dash-dotted line), and for molecule 6 (dotted line); (b) the same as in (a), but for the water oxygen atoms with the dipeptide methyl carbon atoms.

At the interface the alanine dipeptide is surrounded not only by oxygen atoms from water but also from the GMO head groups. Integration of the radial distribution function between oxygen atoms in the dipeptide and the head groups yields a solvation number of 0.4. This yields a total solvation number for the oxygen atoms in the dipeptide of 2.0, a value close to that in aqueous solution. Thus, the loss of hydration around the dipeptide at the interface is almost fully offset by its interactions with the oxygen atoms in GMO head groups. While some differences were observed in the degree to which the five molecules were hydrated, their *total* solvation numbers were almost identical, irrespective of the dipeptide conformation.

The radial distribution functions for molecule 6 differ markedly from those for molecules 1-5. As can be seen from Figure 4, molecule 6 undergoes extensive dehydration. The hydration and total solvation numbers for oxygen atoms of this molecule are 0.9 and 1.3, respectively.

These results provide a link between solvation of the alanine dipeptide at the interface and its conformational preferences. Molecules 1-5 remain in locally polar environments, and their conformational preferences for the α_R and β structures are similar to those of the dipeptide in water. In contrast, molecule 6 looses about 40% of its solvation. This degree of desolvation is similar to that at the water-hexane interface. As a consequence, the conformational preferences of the dipeptide in these two cases are similar. In particular, a considerable population of the C_{7eq} conformation is present in the absence of strong solvation. Desolvation of the polar oxygen atoms of molecule 6 destabilizes this molecule at the interface, However, this is accompanied by a compensating gain in stability resulting from the removal of methyl groups from water.

Arrangement of the Alanine Dipeptide and the Neighboring GMO Molecules at the Interface. In this section we address two questions: (1) what is the orientation of the alanine dipeptide at the interface, and (2) how are the nearby head groups arranged to accommodate the solute? The orientational preferences of the dipeptide can be described by the probability distribution of finding different values of the angles between the normal to the interface and three vectors – the vector from C(1) to C(19), pointing along the backbone of the molecule, and the carbonyl bond vectors C(5)–O(6) and C(15)–O(16), pointing approximately perpendicular to the backbone.

The orientational distribution of the angle between C(1)–C(19) and the normal, shown in Figure 5a, is fairly broad. Even broader distributions are obtained for the other angles, indicating that the dipeptide exhibits considerable orientational freedom at the interface. Orientations in which the dipeptide lies parallel to the interface are preferred, because perpendicular arrangements would require dehydration of one of the oxygen atoms, destablizing these arrangements. Similar preferences were also observed at the water–hexane interface (*15*).

Similar information about the arrangement of the alanine dipeptide at the water–bilayer interface is conveyed by the density profiles of oxygen atoms, nitrogen atoms and methyl groups of this molecule in the direction perpendicular to the interface, shown in Figure 5b. As we can see, they do not differ dramatically from one another. One feature worth noting is that the hydrophobic methyl groups penetrate somewhat deeper into the nonpolar core of the bilayer than do the polar oxygen and nitrogen atoms.

The fact that near the interface the dipeptide does not lie with its hydrophilic groups pointing toward water and its hydrophobic groups pointing toward the membrane can be understood by noting that this molecule is not amphiphilic. Although the dipeptide contains both polar and nonpolar groups, they cannot be segregated into different environments due to constraints imposed by the chemical structure of the molecule. Instead, a compromise is reached, whereby the nonpolar groups are partially removed from water while the polar groups retain most of their solvation shell. This can be accomplished in a relatively broad range of orientations. In contrast, it has been shown both experimentally (*28*) and computationally (*29,30*) that typical amphiphilic molecules, such as phenol and *p*-*n*-pentylphenol, become quite rigid at the water liquid–vapor interface, so that simultaneously the hydrocarbon part is excluded from the water and the polar alcohol group remains hydrated.

When the dipeptide moves into the interfacial region, the GMO molecules must undergo some structural changes to accommodate it. Extensive analysis of the structure of the GMO molecules near the dipeptide reveals that these changes are very subtle. Most of the structural properties of the bilayer, including those of the hydrocarbon core, are not affected by the presence of the dipeptide. It

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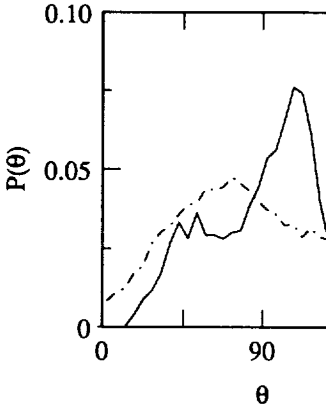

Figure 5. (a) Orientation
vectors C(1)–C(19) (solid
to a vector normal to th
the water phase; (b) Densi
line), oxygen atoms (dash-
the membrane interface. T
the water lies to the right

Dynamics of the Alanin
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(*13*). In that study, it was
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therefore appears that the dipeptide is able to slip into the head group region without causing any major structural perturbations to the bilayer. The transfer of the dipeptide into the interfacial region becomes possible through a series of small adjustments of the orientations and conformations of both the solute and the neighboring GMO molecules. It is possible that this is a generally preferred way in which the bilayer interface interacts with fairly small, neutral species, as large structural perturbations would tend to destablize the bilayer. However, this conclusion does not apply to ions, which induce large changes in the structure of the membrane and the interfacial water (*25*).

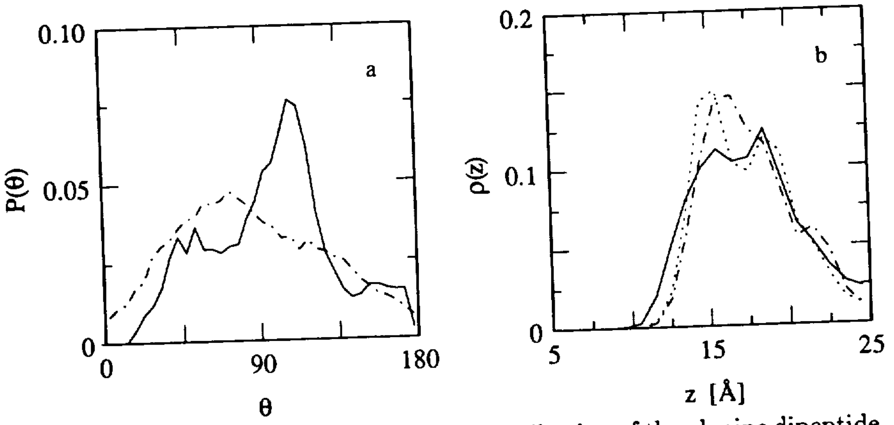

Figure 5. (a) Orientational probability distribution of the alanine dipeptide vectors C(1)-C(19) (solid line), and C=O (dash-dotted line). with respect to a vector normal to the bilayer surface pointing from the bilayer into the water phase; (b) Density profiles for the dipeptide methyl groups (solid line), oxygen atoms (dash-dotted line), and nitrogen atoms (dotted line) at the membrane interface. The center of the bilayer is located at $z = 0$, and the water lies to the right ($z > 16$).

Dynamics of the Alanine Dipeptide at the Interface. The different results on the conformational equilibria of the alanine dipeptide obtained from different MD trajectories indicate that on nanosecond time scales the system is not in equilibrium with respect to slow solvent relaxation. On the other hand, the broad distributions of orientations and positions of the dipeptide along the z-direction of the bilayer suggest that some molecular motions are fairly well equilibrated on the same time scale. The existence of different types of motions in membranes which relax on different time scales has recently been discussed in the context of computer simulations of a dipalmitoylphosphatidylcholine bilayer (*13*). In that study, it was suggested that reorientational dynamics of carbon atoms is fast, while lateral diffusion in the head group region is slow.

Based on our results the following picture may be proposed: The alanine dipeptide is in a "cage" of several neighboring GMO molecules. It possesses considerable orientational and positional freedom as long as its movement requires only reorientation or small structural adjustment of the neighboring GMO molecules. Any large motions which require reorganization of the local environment would be impeded by the GMO "cage". The head group region should be especially rigid while the environment deeper in the bilayer might be more flexible. The dipeptide molecules which penetrate deeper into the bilayer would therefore exhibit more conformational freedom. Note, that the transition between α_R and β conformations is associated with a fairly substantial molecular rearrangement in which ψ changes by about 180°.

One measure of fast dynamics in the system is the average displacement correlation function of the dipeptide. This function is linear on the time scale of 1-5 ps, allowing us to calculate the coefficient of self-diffusion from its slope. In the direction parallel to the bilayer, this coefficient equals 0.05×10^{-5} cm^{2}sec^{-1}. By comparison, the self-diffusion coefficients of the dipeptide in water, hexane and at the water–hexane interface are 0.085, 0.34, and 0.16×10^{-5} cm^{2}sec^{-1}, respectively. Thus, self-diffusion of the alanine dipeptide in all those environments is faster than that at the water-bilayer interface. It appears that solute diffusion at the membrane surface over longer time scales is even slower. This issue is currently being investigated and the results will be published separately.

If the idea that conformational transitions in the alanine dipeptide cause substantial disruptions in the "cage" of the nearby GMO molecules is correct, then a decrease in the rate of isomerization due to solvent effects should be observed. In the transition state theory approximation, every trajectory which reaches the transition state leads to the formation of product. However, when the solvent reorganization lags behind the evolution of the solute along the reaction coordinate, solvent molecules tend to pull the solute back to the reactant state. A convenient way to analyze this effect in isomerization reactions is by calculating the reactive flux correlation function (*31*). A conventional method for obtaining the reactive flux is to monitor a series of short trajectories initiated from the transition state. In the present case, this approach would require additional, extensive calculations. However, by taking advantage of fairly frequent conformational transitions in molecules 5 and 6, we have estimated the dynamic solvent effect from the existing trajectories. The trajectory was monitored for 2 ps after it crossed the transition state. After several recrossings, the system stabilized in either the reactant or the product well. The observation resumed upon the next crossing of the transition state, *etc.* The exact length of a single observation had no effect on the results.

A total of 167 transition events between the α_R and β (or C_{7eq}) conformations were observed, which approximately matches the predictions of transition state theory (*32*). Of these, 76 started in the α_R state and 91 started in the β or C_{7eq} state. During each event, the system crossed the transition state 9 times on average. Only 29 events were reactive, *i.e.* led to the isomerization, yielding a transmission coefficient of 0.17. This value is markedly lower than that obtained in our study of the isomerization of 1,2-dichloroethane at the water–hexane interface (*15*). Thus, as expected, the solvent exerts dynamical effects on the alanine dipeptide and lowers the isomerization rate.

Conclusions

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1990, *29*, 8713–8719.

Conclusions

The alanine dipeptide exhibits a free energy minimum at the water-membrane interface, even though it does not have well-defined hydrophobic and hydrophilic parts and, therefore, should not be considered as amphiphilic. Such interfacial activity appears to be a general feature of molecules which possess sufficient hydrophilicity and lipophilicity. The interface "as seen" by the dipeptide extends over a wide region in which the molecule can move without free energy cost. In contrast, the interfacial region in the water-hexane system is substantially narrower. This is probably due to the fact that in the water-membrane system the environment changes from polar to nonpolar progressively in the head group region while the water-hexane interface is quite sharp.

Over most of the interfacial region the polar groups of the dipeptide remain well solvated by water molecules and the oxygen atoms of the GMO head groups. This probably determines that the α_R and β conformations, which are stable in aqueous solution, are also preferred at the water-membrane interface. Only when the dipeptide penetrates into the bilayer side of the head group region does partial desolvation occur and the C_{7eq} conformation, stabilized by an intramolecular hydrogen bond, becomes significantly populated. A distinguishing effect of the interface is that the free energy barriers to isomerization are considerably reduced compared to those in bulk water and hexane phases.

Accurate determination of conformational equilibria and barriers from molecular dynamics simulations appears to be quite difficult due to slowly relaxing motions in the system which impede equilibration. Their presence was supported by calculations of the dipeptide mobility and dynamic solvent effects on the isomerization reaction. The exact nature of these motions is not well understood but most likely they are associated with collective reorganization of membrane head groups. A better understanding of these slow relaxation phenomena is needed before proceeding to large-scale molecular simulations of longer peptides which exhibit secondary structure at the water-membrane interface.

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