Surface Binding of Alamethicin Stabilizes its Helical Structure: Molecular Dynamics Simulations

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ABSTRACT Alamethicin is an amphipathic α-helical peptide that forms ion channels. An early event in channel formation is believed to be the binding of alamethicin to the surface of a lipid bilayer. Molecular dynamics simulations are used to compare the structural and dynamic properties of alamethicin in water and alamethicin bound to the surface of a phosphatidylcholine bilayer. The bilayer surface simulation corresponded to a loosely bound alamethicin molecule that interacted with lipid headgroups but did not penetrate the hydrophobic core of the bilayer. Both simulations started with the peptide molecule in an α-helical conformation and lasted 2 ns. In water, the helix started to unfold after ~300 ps and by the end of the simulation only the N-terminal region of the peptide remained α-helical and the molecule had collapsed into a more compact form. At the surface of the bilayer, loss of helicity was restricted to the C-terminal third of the molecule and the rod-shaped structure of the peptide was retained. In the surface simulation about 10% of the peptide/water H-bonds were replaced by peptide/lipid H-bonds. These simulations suggest that some degree of stabilization of an amphipathic α-helix occurs at a bilayer surface even without interactions between hydrophobic side chains and the acyl chain core of the bilayer.

INTRODUCTION

Amphipathic α-helices may bind to the surface of a lipid bilayer. Surface-bound α-helices (as opposed to inserted, i.e., transmembrane, α-helices) are important in a number of areas of membrane biology and are found in diverse systems, including (i) antimicrobial peptides such as magainin (Bechinger, 1997) and cecropin (Gazit et al., 1996), (ii) the prepore state of channel-forming peptides, e.g., alamethicin (Sansom, 1993; Cafiso, 1994) and pore-forming toxins, e.g., colicin (Cramer et al., 1995), and (iii) enzymes bound tightly to the surface of a membrane, such as prostaglandin H2 synthase-1 (Picot et al., 1994). An important feature of such peptides is that interaction with a bilayer surface appears to stabilize the α-helical conformation (Vogel, 1987; DeGrado et al., 1989; Chung et al., 1992; Matsuzaki et al., 1994; Bechinger et al., 1993) (also see Sansom (1991) for a review of the earlier literature), i.e., the peptide does not form a stable α-helix in water but does form a stable helix at the bilayer surface. It is this aspect of surface-bound α-helices that we explore in this paper.

Alamethicin (Alm) is a channel-forming peptide that has been intensively investigated using a wide range of experimental and computational approaches (Woolley and Wallace, 1992; Sansom, 1993; Cafiso, 1994). It is 20 residues long with the sequence Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol. It contains multiple α-aminoisobutyric acid (Aib) residues, which stabilize α-helix formation, and a central Gly-X-X-Pro motif, which forms a molecular hinge between the two predominantly α-helical segments. Alm forms an α-helix in membrane mimetic solvents, as shown by nuclear magnetic resonance studies in methanol (Esposito et al., 1987; Gibbs et al., 1997) and by crystals obtained from a methanol/acetonitrile solution (Fox and Richards, 1982). It is able to bind to the surface of a lipid bilayer (Dempsey and Handcock, 1996) but may also insert itself into a bilayer to adopt a transmembrane orientation (North et al., 1995; Jayasinghe et al., 1998). Which orientation is preferred depends on, among other things, the peptide:lipid molar ratio, the degree of hydration of the bilayer, the temperature, and the nature of the lipid (Vogel, 1987; Huang and Wu, 1991; He et al., 1996).

Surface binding of Alm is believed to be the first step toward channel formation. The generally accepted model is that Alm helices bind to and are stabilized at the bilayer surface. Subsequent insertion of these helices is aided by the application of a transmembrane voltage difference. Inserted helices then self-assemble into bundles that form pores through which ions may flow. To fully understand the mechanism of pore formation by Alm, one needs atomic resolution simulations of Alm at the bilayer surface, of single Alm helices spanning a bilayer (Tieleman et al., 1999c), and of bundles of Alm helices (Tieleman et al., 1999a,b), in addition to a description of the forces driving helix insertion and aggregation.

In this paper we describe a 2-ns molecular dynamics (MD) simulation of Alm loosely bound at the surface of a phosphatidylcholine bilayer and compare the secondary structure stability of the helix with that in a 2-ns MD simulation of Alm in water.

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METHODS

MD simulations were conducted as described previously (Tieleman et al., 1999c). Simulations were carried out for the following systems: Alm in a box of 3467 water molecules plus one Na^+ ion (henceforth *Alm/water*), giving a total of 10,569 atoms, and Alm at the surface of a bilayer of 128 palmitoyloleoylphosphatidylcholine (POPC) molecules plus one Na^+ ion and 3552 water molecules (henceforth *Alm/surface*), giving a total of 17,480 atoms. For *Alm/water* the initial system was generated by placing the Alm helix in a 4.9-nm^3 box of water and then solvating and adding a Na^+ ion. This simulation was an extension of an earlier 1-ns simulation (Tieleman et al., 1999c).

For *Alm/surface* a fully equilibrated POPC bilayer of 128 lipid molecules was used. An Alm helix was placed above the bilayer with its center at $z = 1.4$ nm (the bilayer center being at $z = 4.0$ nm) such that it was close to, but not in contact with, the bilayer interface. The helix was oriented such that its long axis was perpendicular to the bilayer normal, with its hydrophilic side chains (Gln7, Glu18, and Gln19) pointing away from the bilayer surface. This system (peptide plus bilayer) was solvated and a single Na^+ ion added. After energy minimization, an MD simulation was performed in which an acceleration along the bilayer normal (0.1 nm ps^{-2}) was applied to the peptide. This moved the helix closer to the bilayer while allowing the lipid headgroups to relax in response to the approaching peptide. Analysis of the potential energy of the system showed a plateau after ~300 ps, so the structure at 350 ps was saved. This was used to set up the *Alm/surface* starting configuration by replacing the Alm helix with its initial, unperturbed structure and then resolvating. This yielded a system in which the lipids had relaxed in response to the surface Alm, but with the same Alm starting structure as in the *Alm/water* simulation. The resulting system was energy-minimized and a 2-ns MD run was performed. MD simulations were run using GROMACS (http://rugmd0.chem.rug.nl/~gmx/gmx.html), as described previously (Tieleman et al., 1999c). Analysis was performed using GROMACS and secondary structure analysis employed DSSP (Kabsch and Sander, 1983).

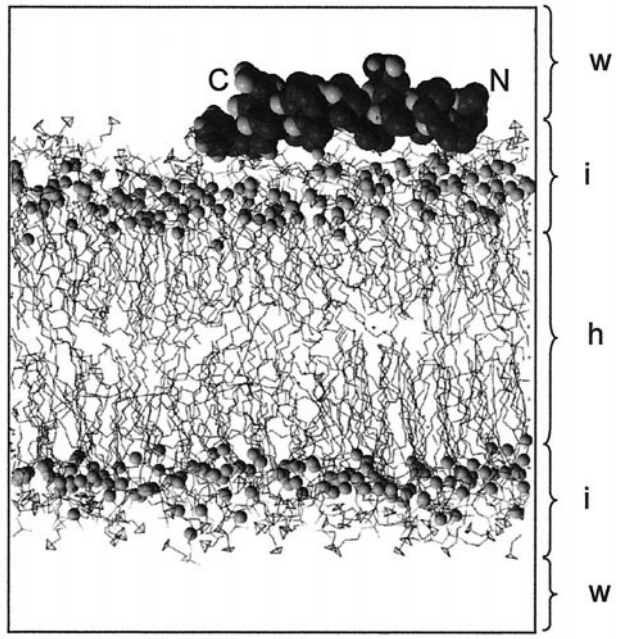

FIGURE 1 Snapshot at $t = 0$ of the *Alm/surface* simulation system. The water molecules on either side of the POPC bilayer are omitted for clarity. The carbonyl atoms of the lipid headgroups, marking the farthest extent of penetration of water molecules into the bilayer, are shown as small gray spheres. The approximate extents of the bulk water (w), interfacial (i), and hydrophobic core (h) regions are indicated.

RESULTS

The initial configuration of the *Alm/surface* simulation system is shown in Fig. 1. This loose complex represents that formed, e.g., on the first encounter of a helical Alm molecule with the bilayer surface. It does not preclude the possibility of subsequent formation of a tight complex in which the Alm molecule is drawn more deeply into the interfacial region with the hydrophobic side chains penetrating the hydrophobic core.

An overall measure of the stability of the Alm helix in the two environments was obtained by comparing the time-dependent Cα atoms' root mean square deviations (RMSDs) from the starting structure (Fig. 2 *A*). For *Alm/water* there is a large overall change in structure, with a marked jump in the Cα RMSD at ~1.5 ns. This contrasts with the *Alm/surface* simulation, in which the overall change in RMSD is from ~0.18 nm at the start of the simulation to ~0.28 nm at the end. Note that this latter value of the RMSD is somewhat more than that for a single Alm helix in methanol or when spanning a lipid bilayer (Tieleman et al., 1999c). Thus, the RMSDs suggest that the Alm helix is significantly more stable in *Alm/surface* than in *Alm/water*.

Changes in structure may be visualized as snapshots of Cα traces of Alm taken every 200 ps (Fig. 2, *B* and *C*). Over the course of the 2-ns *Alm/water* simulation the N-terminal segment (residues 1 to 10) retains its initial α-helical conformation. There is considerable conformational change about the Gly-X-X-Pro hinge and in the C-terminal segment. In particular, in the latter half of the simulation the Alm molecule folds back on itself such that by the end of the simulation, the C-terminus is only 1.0 nm away from the N-terminus (Cα 1 to Cα 20), compared to 2.8 nm in the initial α-helical structure. This behavior contrasts with that of the *Alm/surface* simulation. Although there is evidence for some conformational flexibility about the Gly-X-X-Pro hinge in *Alm/surface*, this is much more limited and the molecule remains rod-shaped and predominantly helical.

Analysis of secondary structure in the two simulations confirms this impression (Fig. 3). For *Alm/water*, loss of α-helicity at the C-terminus begins at ~0.2 ns. This propagates back up the polypeptide chain such that by 1.5 ns, about two-thirds of the chain has lost its α-helical conformation. In contrast, in the *Alm/surface* simulation α-helicity is retained in residues 1–12 throughout 2 ns. Loss of helicity is confined to the residues C-terminal to the Gly-X-X-Pro hinge. The extent of loss of α-helicity in *Alm/surface* is between that of *Alm/water* and that of isolated Alm helices in either methanol or spanning a POPC bilayer (Tieleman et al., 1999c). Thus, the bilayer surface appears able to stabilize the α-helical conformation of Alm relative to bulk aqueous solution, at least on a 2-ns timescale.

A picture of the location of the Alm molecule relative to the lipid headgroups and water molecules that make up the water/bilayer interface is provided by the density profiles

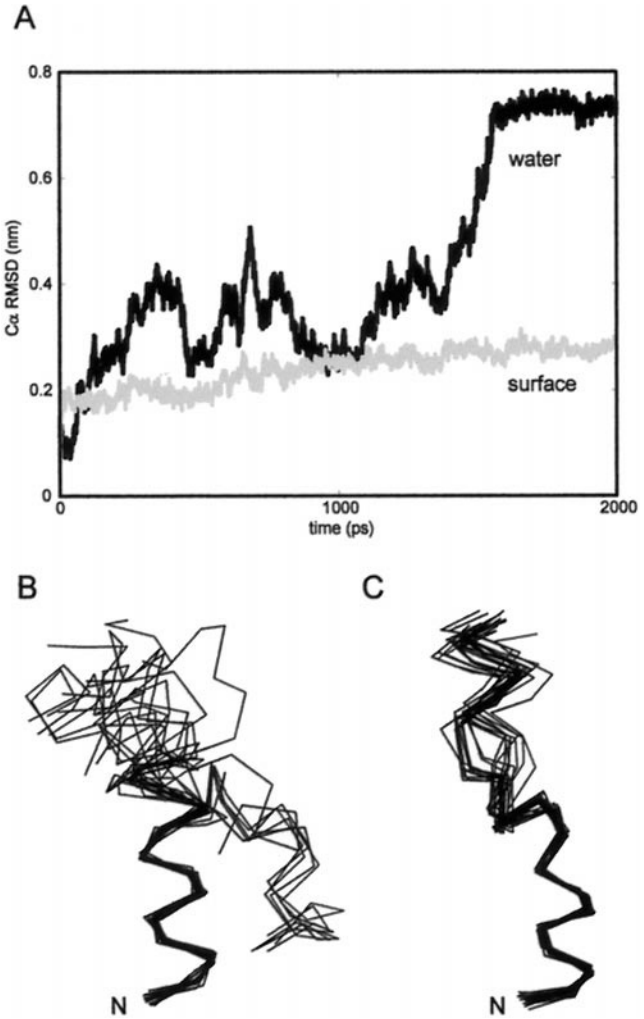

FIGURE 2 (*A*) RMSDs versus time for the Cα atoms in the two simulations: *Alm/water* (*black line*) and *Alm/surface* (*gray line*). Cα traces, corresponding to structures saved every 200 ps, for (*B*) *Alm/water*; and (*C*) *Alm/surface*. In each case the N-terminal helices (residues 1 to 10) were superimposed.

along the bilayer normal for Alm, POPC, and water (Fig. 4 *A*). Alm is located on the edge of the water/bilayer interfacial region. Thus the Alm molecule is *not* fully exposed to bulk water even though the *Alm/surface* simulation corresponds to a loose complex. This location of alamethicin relative to the bilayer contrasts with the models of, e.g., Wiener and White (1992), White (1994), Chung et al. (1992), and Lear et al. (1994), in which surface-bound helices are proposed to be closer to the hydrophobic core of the bilayer. These latter models may correspond to a tight complex.

The interactions of Alm with the bilayer surface were examined in more detail by counting peptide/lipid contacts and H-bonds as a function of time (Fig. 4, *B* and *C*). Both analyses reveal a degree of tightening of peptide/lipid interactions during the first ~200 ps. Over the second half of the simulation the mean number (and standard deviation) of peptide/lipid contacts is 14 (± 3). The mean number of H-bonds from Alm to the POPC headgroups is 4 (± 1). Almost all of these H-bonds are from peptide backbone NH (or C-terminal −OH) H-atoms to phosphate oxygen atoms rather than to glycerol or acyl group oxygens of the lipids. The number of peptide/water H-bonds for *Alm/surface* is 38 (± 4), compared to 41 (± 4) for *Alm/water*, both figures averaged over the last 1 ns of the respective simulations. Thus the total number of H-bonds to the peptide is conserved, but in *Alm/surface* ~10% of the peptide/water H-bonds are replaced by peptide/lipid H-bonds.

DISCUSSION

The primary conclusion from the simulation results described in this paper is that the α-helical conformation of a membrane-active peptide is stabilized when the peptide is loosely bound to the surface of a lipid bilayer, even though the more hydrophobic surface of the helix does not penetrate into the bilayer core. This is relevant to models of how amphipathic helices are stabilized at water/bilayer interfaces. The traditional view (Sansom, 1998) is that α-helix stabilization is due to partitioning of hydrophilic and hydrophobic side chains between the aqueous phase and the hydrophobic core of the bilayer, respectively. This requires the helix to be located deep in the interfacial region, as in the models of White (1994) and Lear et al. (1994). Although this may be so in tighter peptide:bilayer complexes, it is not so in the loose complex described here. Thus, the apparent stabilization in the current simulation must arise via a different mechanism. It is significant that results similar to those described here for Alm have been obtained for loose complexes of other membrane active peptides, e.g., dermaseptin (LaRocca and Sansom, unpublished results) and melittin (Baumgärtner and Lin, personal communication), with lipid bilayers. Thus, this may be a general phenomenon meriting further theoretical and experimental studies of the nature of the interactions of amphipathic helices with surfaces. We note with interest that, e.g., White (1999) has emphasized the complex nature of the interfacial region of bilayers and of the interactions of amphipathic helices with this region.

A simulation of the interactions of melittin with a dimyristoylphosphatidylcholine bilayer has been published (Bernèche et al., 1998). In this study, the initial configuration of the system was such that although the overall helix axis was approximately parallel to the bilayer plane, the helix kink (induced by the central proline) caused the N-terminus of the helix to penetrate deep into the core of the bilayer. The α-helical conformation of melittin remained stable throughout the 1-ns simulation. Thus, this simulation is one of a tight complex between peptide and bilayer in which the peptide is largely buried in the hydrophobic core of the bilayer.

Why is an Alm α-helix more stable in the outer half of the interfacial region than in bulk water? Simulation studies of pure lipid bilayers (reviewed by, e.g., Tieleman et al.,

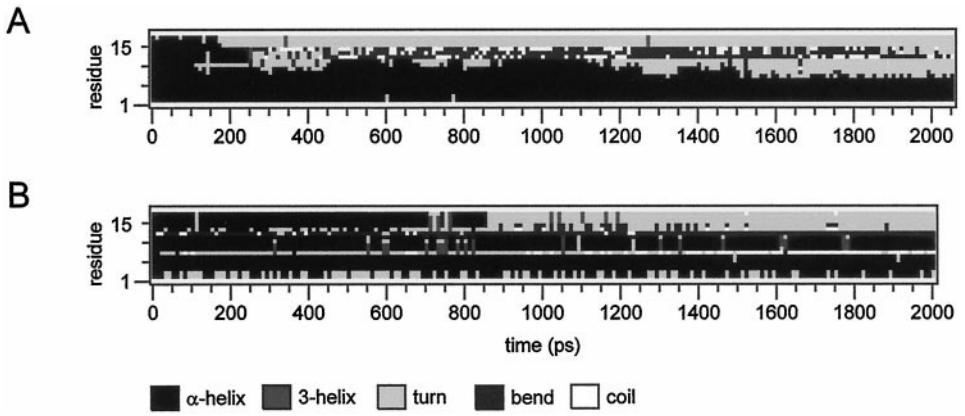

FIGURE 3 Secondary structure, analyzed using DSSP (Kabsch and Sander, 1983), as a function of time for (*A*) *Alm/water* and (*B*) *Alm/surface*. Grayscale: black, α-helix; dark gray, bend; pale gray, turn; and white, coil, as indicated at the foot of the diagram.

(1997) have shown that water in the interfacial regions differs in dynamic and orientational properties from bulk water. In particular, such water is less mobile ($D \sim 0.2D_{bulk}$, where D is the self-diffusion coefficient of water in the interface and D_{bulk} is that of bulk water) and is nonrandomly oriented relative to the bilayer normal (Marrink and Berendsen, 1994). The altered properties of water in the interfacial region may help to explain why it does not promote the unfolding of Alm to the same extent as bulk water. There are two possibilities. First, the free energy cost of breaking a lipid/water H-bond may outweigh that of forming a peptide/water H-bond, thus stabilizing the peptide. Second, there may be kinetic effects, i.e., unfolding is much slower in the interfacial region than in bulk water.

It is worthwhile to consider the limitations of our simulations. Besides the usual reservations concerning the use of electrostatic cutoffs, addressed in detail in, e.g., Tieleman et al. (1997) and Tobias et al. (1997), there remain considerations specific to simulations of a peptide bound to the surface of a lipid bilayer. The major concern is the way in which the initial configuration of the *Alm/surface* simulation was generated. Continuum electrostatics calculations of melittin close to a bilayer showed that the free energy profile is rather flat (Bernèche et al., 1998). Although such calculations ignore most atomic details, they do indicate that melittin and probably other membrane-binding peptides adopt a large range of possible orientations, consistent with a wide range of experimental evidence. We gave the peptide an acceleration toward the bilayer to generate a number of structures with the peptide at different distances from the bilayer and then used a structure of a loose complex between the peptide and bilayer as a starting structure for our simulation. This is just one orientation and it is unlikely that the peptide will adopt a tightly bound, very different orientation in 2 ns. From the current simulations in water and of one complex, we cannot estimate any free energies. Ideally, one would perform a systematic series of simulations of the peptide at different distances from and in different orientations to the bilayer to obtain a complete picture of the binding of alamethicin to a bilayer. We are currently attempting such a more systematic approach but it requires at least an order of magnitude more computational effort.

We do not think that within the current timescale accessible by MD it is possible to see a transition from a loose to a tight complex, even though such a transition may occur experimentally. Even on a much simpler interface during several hundred nanoseconds' simulation of a small peptide, only limited equilibration was observed (Chipot and Pohorille, 1997, 1998). Alamethicin will bind to a bilayer and in doing so will probably assume a whole range of orientations, including the one we looked at. Free energy calculations on melittin suggested that there are no clear minima or maxima for orientation of melittin on a bilayer, although those calculations used a continuum electrostatics method with more severe approximations than are used in MD.

Unfortunately, we cannot say if the free energy of the alamethicin is higher in water than on the surface, at least not from the current simulations. Estimating free energy costs from MD is theoretically possible and has frequently been used for relatively small changes. Usually either thermodynamic integration along a changing Hamiltonian or some form of umbrella sampling is used. In this particular case it would be very complicated to estimate free energies because it is not clear which situations should be compared. Estimating the free energy of alamethicin as function of depth or orientation in the bilayer from simulations is extremely hard and will require simulations several orders of magnitude longer, if it is possible at all. However, as alamethicin is largely helical (Dempsey and Handcock, 1996) when bound to a bilayer, we would not expect the peptide to unfold completely if we simulated for a longer time.

We should also comment on the use of an acceleration to make the starting structure. We do not use a free energy criterion to determine the starting structure but simply select a structure from the accelerated simulation run in which the distance from the bilayer corresponds to the peptide present in the interfacial region. It may prove not so important exactly how this structure was created. For example, we could have simply docked the peptide into the same position, although this would have generated some unfavorably close lipid/peptide contacts that would had to have been relaxed. Thus, the acceleration is simply a useful trick to generate a series of starting structures with the peptide at different locations with respect to the bilayer. As mentioned

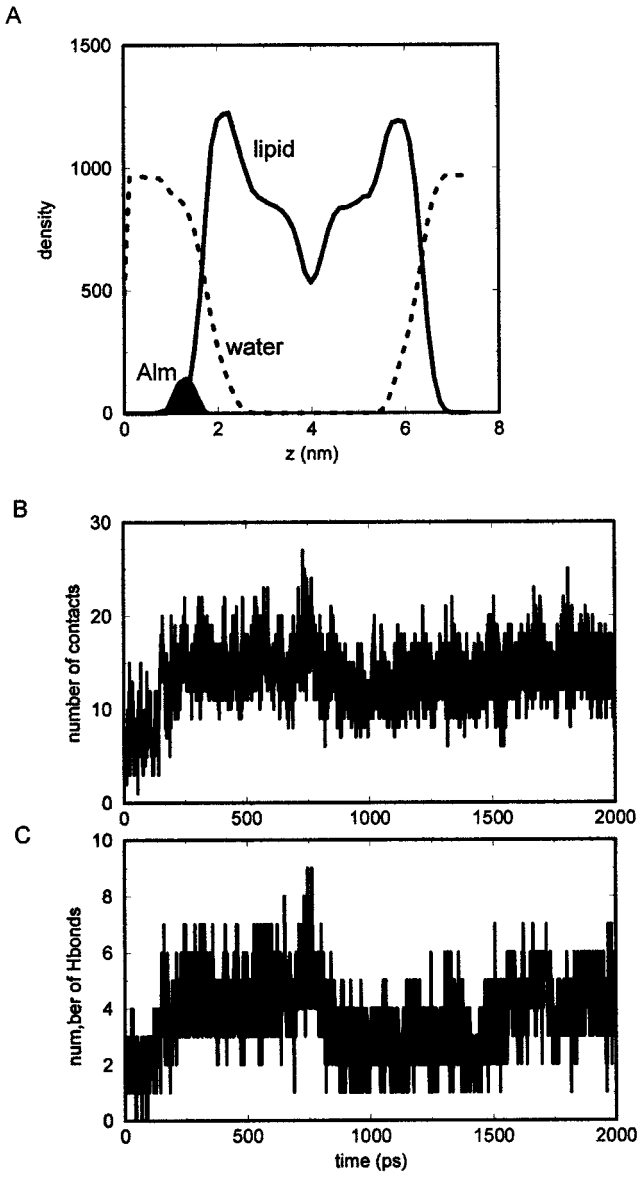

FIGURE 4 (*A*) Density profiles calculated along the bilayer normal for the *Alm/surface* simulation. The lipid density profile is shown with a solid line and the water profile with a broken line. The Alm density profile is shown as the filled black region. (*B, C*) Analysis of lipid/Alm interactions. *A* shows the number of peptide atom to lipid atom contacts of less than 0.3 nm as a function of time. *B* shows the number of H-bonds between Alm atoms and POPC headgroup atoms.

above, it would be highly desirable to develop a more systematic way of searching orientations and distances from a bilayer.

Systematic studies of alternative starting configurations of peptide helices at bilayer surfaces are not the only challenge for the future. Peptides such as Alm are believed to alter their position, orientation, and/or conformation at a bilayer/water interface when a transbilayer voltage difference of ~100–200 mV is applied. Although there have been preliminary studies of how to include a transbilayer voltage in simulations (Biggin and Sansom, 1996; Biggin et al., 1997; Roux, 1997), further methodological work is required before such methods may be applied to prolonged simulations of peptide/water/bilayer systems.

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REFERENCES

Bechinger, B. 1997. Structure and function of channel-forming peptides: magainins, cecrpins, melittin and alamethicin. *J. Membr. Biol.* 156: 197–211.

Bechinger, B., M. Zasloff, and S. J. Opella. 1993. Structure and orientation of the antibiotic peptide magainin in membranes by solid-state nuclear magnetic resonance spectroscopy. *Protein Sci.* 2:2077–2084.

Bernèche, S., M. Nina, and B. Roux. 1998. Molecular dynamics simulation of melittin in a dimyristoylphosphatidylcholine bilayer membrane. *Biophys. J.* 75:1603–1618.

Biggin, P., J. Breed, H. S. Son, and M. S. P. Sansom. 1997. Simulation studies of alamethicin-bilayer interactions. *Biophys. J.* 72:627–636.

Biggin, P. C., and M. S. P. Sansom. 1996. Simulation of voltage-dependent interactions of α-helical peptides with lipid bilayers. *Biophys. Chem.* 60:99–110.

Cafiso, D. S. 1994. Alamethicin: a peptide model for voltage gating and protein membrane interactions. *Ann. Rev. Biophys. Biomol. Struct.* 23: 141–165.

Chipot, C., and A. Pohorille. 1997. Structure and dynamics of small peptides at aqueous interfaces: a multi-nanosecond molecular dynamics study. *J. Mol. Struct. (Theochem.)* 398–399:529–535.

Chipot, C., and A. Pohorille. 1998. Structure and dynamics of small peptides at aqueous interfaces: a multi-nanosecond molecular dynamics study. *J. Am. Chem. Soc.* 120:11912–11924.

Chung, L. A., J. Lear, and W. F. DeGrado. 1992. Fluorescence studies of the secondary structure and orientation of a model ion channel peptide in phospholipid vesicles. *Biochemistry.* 31:6608–6616.

Cramer, W. A., J. B. Heymann, S. L. Schendel, B. N. Deriy, F. S. Cohen, P. A. Elkins, and C. V. Stauffacher. 1995. Structure-function of the channel-forming colicins. *Ann. Rev. Biophys. Biomol. Struct.* 24:611–641.

DeGrado, W. F., Z. R. Wasserman, and J. D. Lear. 1989. Protein design, a minimalist approach. *Science.* 243:622–628.

Dempsey, C. E., and L. J. Handcock. 1996. Hydrogen-bond stabilities in membrane-reconstituted alamethicin from amide-resolved hydrogen-exchange measurements. *Biophys. J.* 70:1777–1788.

Esposito, G., J. A. Carver, J. Boyd, and I. D. Campbell. 1987. High resolution ^{1}H NMR study of the solution structure of alamethicin. *Biochemistry.* 26:1043–1050.

Fox, R. O., and F. M. Richards. 1982. A voltage-gated ion channel model inferred from the crystal structure of alamethicin at 1.5 Å resolution. *Nature.* 300:325–330.

Gazit, E., I. R. Miller, P. C. Biggin, M. S. P. Sansom, and Y. Shai. 1996. Structure and orientation of the mammalian antibacterial peptide cecropin P1 within phospholipid membranes. *J. Mol. Biol.* 258: 860–870.

Gibbs, N., R. B. Sessions, P. B. Williams, and C. E. Dempsey. 1997. Helix bending in alamethicin: molecular dynamics simulations and amide hydrogen exchange in methanol. *Biophys. J.* 72:2490–2495.

He, K., S. J. Ludtke, W. T. Heller, and H. W. Huang. 1996. Mechanism of alamethicin insertion into lipid bilayers. *Biophys. J.* 71:2669–2679.

Huang, H. W., and Y. Wu. 1991. Lipid-alamethicin interactions influence alamethicin orientation. *Biophys. J.* 60:1079–1087.

Jayasinghe, S., M. Barranger-Mathys, J. F. Ellena, C. Franklin, and D. S. Cafiso. 1998. Structural features that modulate the transmembrane migration of a hydrophobic peptide in lipid vesicles. *Biophys. J.* 74: 3023–3030.

Kabsch, W., and C. Sander. 1983. Dictionary of protein secondary structure:- pattern-recognition of hydrogen-bonded and geometrical features. *Biopolymers.* 22:2577–2637.

Lear, J. D., Z. R. Wasserman, and W. F. DeGrado. 1994. Use of synthetic peptides for the study of membrane protein structure. *In* Membrane Protein Structure: Experimental Approaches. S. H. White, editor. Oxford University Press, Oxford. 335–354.

Marrink, S. J., and H. J. C. Berendsen. 1994. Simulation of water transport through a lipid membrane. *J. Phys. Chem.* 98:4155–4168.

Matsuzaki, K., O. Murase, H. Tokuda, S. Funakoshi, N. Fujii, and K. Miyajima. 1994. Orientational and aggregational states of magainin 2 in phospholipid bilayers. *Biochemistry.* 33:3342–3349.

North, C. L., M. Barranger-Mathys, and D. S. Cafiso. 1995. Membrane orientation of the N-terminal segment of alamethicin determined by solid-state ^{15}N NMR. *Biophys. J.* 69:2392–2397.

Picot, D., P. J. Loll, and M. Garavito. 1994. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase 1. *Nature.* 367: 243–249.

Roux, B. 1997. Influence of the membrane potential on the free energy of an intrinsic protein. *Biophys. J.* 73:2980–2989.

Sansom, M. S. P. 1991. The biophysics of peptide models of ion channels. *Prog. Biophys. Mol. Biol.* 55:139–236.

Sansom, M. S. P. 1993. Structure and function of channel-forming peptaibols. *Quart. Rev. Biophys.* 26:365–421.

Sansom, M. S. P. 1998. Peptides and lipid bilayers:- dynamic interactions. *Curr. Opin. Colloid Interface Sci.* 3:518–524.

Tieleman, D. P., H. J. C. Berendsen, and M. S. P. Sansom. 1999a. An alamethicin channel in a lipid bilayer: molecular dynamics simulations. *Biophys. J.* 76:1757–1769.

Tieleman, D. P., J. Breed, H. J. C. Berendsen, and M. S. P. Sansom. 1999b. Alamethicin channels in a membrane: molecular dynamics simulations. *Faraday Disc.* (in press).

Tieleman, D. P., S. J. Marrink, and H. J. C. Berendsen. 1997. A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems. *Biochim. Biophys. Acta.* 1331:235–270.

Tieleman, D. P., M. S. P. Sansom, and H. J. C. Berendsen. 1999c. Alamethicin helices in a bilayer and in solution: molecular dynamics simulations. *Biophys. J.* 76:40–49.

Tobias, D. J., K. C. Tu, and M. L. Klein. 1997. Atomic-scale molecular dynamics simulations of lipid membranes. *Curr. Opin. Coll. Interface Sci.* 2:15–26.

Vogel, H. 1987. Comparison of the conformation and orientation of alamethicin and melittin in lipid membranes. *Biochemistry.* 26:4562–4572.

White, S. H. 1999. Protein folding on membranes: mechanism of secondary structure formation. *Biophys. J.* 76:A4.

White, S. N. 1994. Hydropathy plots and the prediction of membrane protein topology. *In* Membrane Protein Structure: Experimental Approaches. S. N. White, editor. Oxford University Press, New York.

Wiener, M. C., and S. H. White. 1992. Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of X-ray and neutron diffraction data. III. Complete structure. *Biophys. J.* 61: 434–447.

Woolley, G. A., and B. A. Wallace. 1992. Model ion channels: gramicidin and alamethicin. *J. Membr. Biol.* 129:109–136.