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ARTICLE

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Implicit solvent model estimates of the stability of model structures of the alamethicin channel

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Abstract Alamethicin is a hydrophobic helical peptide of 20 residues, which oligomerizes to form ion-conducting channels in membranes. The behavior of an intact alamethicin channel in POPC bilayers was recently studied, using 2 ns molecular dynamics (MD) simulations of a model hexameric channel. These simulations produced numerous conformations of the channel. In the present study, we used 11 of these channel conformations and carried out continuum-solvent model calculations, similar to those used for the monomers in our previous studies, to investigate the energetics of the channel inside the lipid bilayer. Our results suggest that, out of the 11 channel conformations produced by the MD simulations, only four are stable inside the lipid bilayer, with water-to-membrane free energies of transfer ranging from ~−6 to ~−10 kcal/mol. Analysis of the results suggests two causes for the apparent instability of the remainder of the structures inside the lipid bilayer, both resulting from the desolvation of channel polar groups (i.e. their transfer from the aqueous phase into the bilayer). The first is specific, uncompensated backbone hydrogen bonds, which exist in the region of the channel exposed to the hydrocarbon of the lipid bilayer. The second is exposure of intra-pore water molecules to the surrounding lipid. Thus, the association of these structures with the membrane involves a large electrostatic desolvation free-energy penalty. The apparent conflict between continuum-solvent and MD calculations, and its significance for the interpretation of membrane proteins simulations, are discussed.

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Introduction

Alamethicin is a 20 amino acid antibiotic peptide, which self-assembles to form ion channels in lipid bilayers. The peptide is predominantly α-helical in solution (Fox and Richards 1982; Banerjee and Chan 1983; Esposito et al. 1987; Yee and O'Neil 1992), and the helical structure of its N-terminus is maintained in dimyristoylphosphatidylcholine (DMPC) (North et al. 1995). Based on single-channel measurements (Gordon and Haydon 1975), it has been suggested that alamethicin channels of different sizes are formed by a different number of monomers (estimated between four and 11), depending on the magnitude of the membrane voltage (Sansom 1993). The small size of alamethicin makes it an attractive model for the study of both peptide–membrane interactions and the various aspects of protein channel structure and function. Indeed, alamethicin has been extensively studied, both theoretically and experimentally (reviewed by Cafiso 1994; Sansom 1998).

Early theoretical studies of the intact alamethicin channel have focused primarily on the interpretation of the experimentally observed current–voltage curves and the conductance behavior of single and multiple channels (e.g. Baumann and Mueller 1974; Boheim 1974). Theoretical investigation into the details of the channel–membrane interactions has only recently been undertaken, with the main contribution coming from molecular dynamics (MD) simulations (reviewed by Tieleman and Sansom 2001).

Breed et al. (1996) used MD simulations in vacuo to generate alamethicin channel models composed of five to nine monomers, and to study the dynamics of water molecules inside the transmembrane pore of the channel. The results indicated that the dipole moments of the

intra-pore water molecules were aligned antiparallel to the dipoles of the channel helices, and that the diffusion of these water molecules was reduced in comparison to the diffusion of bulk water. Similar simulations were carried out in a later study (Breed et al. 1997b) that addressed additional aspects of channel dynamics, such as the role of Gln^7 hydrogen-bonding and backbone solvation in the stabilization of the open state of the channel.

Recently, Tieleman et al. (1999) carried out 2 ns MD simulations of the alamethicin channel in a full POPC lipid bilayer, using the in vacuo-generated model of the hexameric channel (Breed et al. 1997a). The calculations addressed dynamic aspects of the channel, focusing on the structure of the α-helices of the channel, and on their short-term interactions with each other, the lipid bilayer, and the water molecules inside the pore. Overall, the model channel was found to be stable throughout the simulation.

The present work is complementary to that of Tieleman et al. (1999). We used continuum-solvent model calculations to study the long-term stability of the model alamethicin channel of Tieleman et al. (1999) in the lipid bilayer. The calculations involved 11 representative structures, taken as snapshots from the simulation. The results suggest that only four of the 11 structures were stable inside the lipid bilayer. Atomic-based analysis of the results also suggests that the apparent instability of the rest of the model structures results from the exposure of either uncompensated backbone hydrogen bonds or intra-pore water molecules to the surrounding lipid membrane.

This study demonstrates an apparent discrepancy in the prediction of channel stability in the membrane by MD simulations and continuum-solvent model calculations. The discrepancy is discussed in the light of the differences between explicit (all-atom) and mean-field (implicit solvent model) calculations, which constitute the major approaches in the computational investigation of protein–membrane interactions.

Methods

The exact details concerning the formation of the alamethicin channel in the lipid bilayer are unknown, although the experimental data (e.g. Barranger-Mathys and Cafiso 1994) may be interpreted in a way that suggests a two-step process, which includes the insertion of each monomer, and their subsequent oligomerization inside the membrane. For convenience, we considered a hypothetical thermodynamic process, in which the separate alamethicin monomers first assume an ordered conformation in the aqueous solution. They are then assembled to form the intact channel, which is inserted into the lipid bilayer (Fig. 1).

In accordance with the virtual path presented above, we broke down the total free energy difference between the alamethicin channel in the membrane and its separate monomers in the aqueous phase (ΔG_{tot}) into a sum of differences of the following terms: the free energy resulting from conformational effects in the monomers (ΔG_{con}); the free energy of channel assembly in water (ΔG_{assem}); and the free energy of transfer of the rigid channel from the aqueous solution into the lipid bilayer (ΔG_{trans}):

$$\Delta G_{tot} = \Delta G_{con} + \Delta G_{assem} + \Delta G_{trans} \quad (1)$$

ΔG_{trans} includes the electrostatic (ΔG_{elc}) and nonpolar (ΔG_{np}) contributions to the solvation free energy ($\Delta G_{sol} = \Delta G_{elc} + \Delta G_{np}$), channel immobilization effects (ΔG_{imm}), lipid perturbation effects (ΔG_{lip}), and membrane deformation effects (ΔG_{def}) (Engelman and Steitz 1981; Jacobs and White 1989; Fattal and Ben-Shaul 1993; Milik and Skolnick 1993; Ben-Tal et al. 1996; White and Wimley 1999; Kessel and Ben-Tal 2002):

$$\Delta G_{trans} = \Delta G_{sol} + \Delta G_{imm} + \Delta G_{lip} + \Delta G_{def} \quad (2)$$

The methodology for evaluating each of the terms in Eq. (2) has been described in detail in our studies of the interactions of alamethicin monomers with lipid bilayers (Kessel et al. 2000a, 2000b). Here we give only a brief overview, with emphasis on the modifications we made to account for the oligomeric channel.

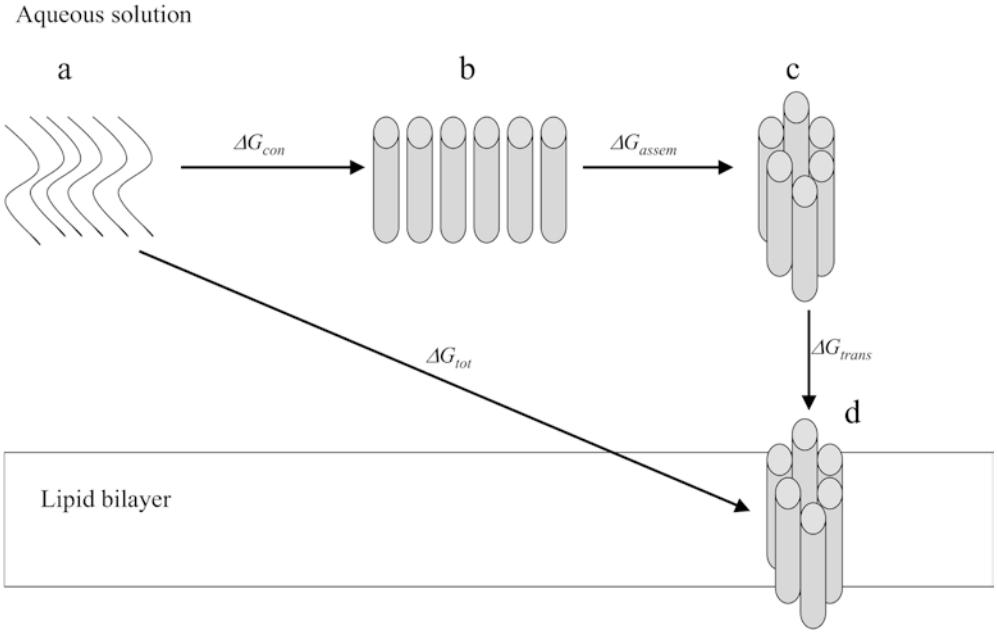


Fig. 1 A hypothetical thermodynamic cycle, describing the formation of the alamethicin channel in the lipid bilayer. The six alamethicin monomers first assume an ordered (helical) conformation in the aqueous solution (*a* to *b*), then assemble to form an oligomeric channel (*b* to *c*), and finally insert into the lipid bilayer (*c* to *d*). These steps, respectively, are accompanied by the following free energy changes (Eq. 1): the conformational free energy (ΔG_{con}), the free energy of channel assembly (ΔG_{assem}), and the free energy of transfer of the channel into the lipid bilayer (ΔG_{trans})

Estimation of ΔG_{con} and ΔG_{assem}

ΔG_{con} is the free energy change resulting from conformational changes in six (separated) molecules of monomeric alamethicin during the transfer of the channel from the aqueous solution into the lipid bilayer. For convenience, in the hypothetical thermodynamic cycle of Fig. 1, the conformational changes take place in the aqueous solution prior to the transfer into the lipid bilayer.

ΔG_{assem} is the free energy change due to the hypothetical assembly of the six monomers in the aqueous phase to form the correct channel structure. These changes result, for example, from monomer–monomer and monomer–water interactions, desolvation of peptide regions involved in these interactions, loss of entropy of both the peptide and water, and burial of hydrophobic areas.

It is very challenging to calculate the values of ΔG_{con} and ΔG_{assem} (Brooks 1998; Guerois and Serrano 2001). Unfortunately, our current model (see below) does not allow us to calculate or estimate these contributions, and they were hence neglected. Still, one might argue that these contributions are unfavorable. Both experimental and computational studies (see review by Sansom 1993) show that alamethicin monomers tend to be, at least partially, disordered in the aqueous solution, which makes their folding unfavorable. ΔG_{assem} might also be unfavorable for entropic reasons. Owing to the hydrophobic nature of alamethicin, it is expected to aggregate in solution, but is still unlikely to assemble to form specific channel structures. Conversely, inside the lipid bilayer, the formation of channel structures is more plausible, since the lipid environment induces the formation of secondary structures in the monomers, which have two effects. First, the monomers have fewer internal degrees of freedom, which decreases the number of possible nonspecific aggregates. The presence of the lipid bilayer around the monomers also decreases their external degrees of freedom, again limiting the number of nonspecific aggregates. Secondly, the formation of secondary structures in the monomers promotes specific monomer–monomer interactions, which underlie the structure of the functional channel.

In summary, we cannot calculate ΔG_{con} and ΔG_{assem} but propose that they are probably small and positive, which means that insertion of the channel into the lipid bilayer is possible only when these terms are overcompensated by negative ΔG_{trans} contributions.

Calculation of ΔG_{sol}

ΔG_{sol} is the free energy of transfer of alamethicin at a given (rigid) channel conformation from water to a bulk hydrocarbon phase. It accounts for electrostatic contributions (ΔG_{elc}) resulting from changes in the solvent's polarity, as well as for nonpolar (hydrophobic) effects (ΔG_{np}), which result from both differences in the van der Waals interactions of alamethicin with the hydrocarbon and aqueous phases, and from solvent structure effects.

Three-dimensional model structures of the alamethicin channel were produced by 2 ns MD simulation (Tieleman et al. 1999), and ΔG_{sol} was calculated using continuum solvent models as in Kessel et al. (2000a, 2000b). The six alamethicin monomers constituting the channel and the water molecules inside the pore were represented in atomic detail, with atomic radii and partial charges defined at the coordinates of each nucleus.

The radii and charges assigned to the peptide were taken from PARSE (Sitkoff et al. 1994, 1996), which has been derived to reproduce the partitioning of solutes between water and a low-dielectric environment. In our previous studies of the association of various peptides, including alamethicin monomers with lipid bilayers, calculations based on PARSE have yielded values that were in very good agreement with experimental data. The charges of oxygen and hydrogen atoms of water molecules, which were missing in PARSE, have been assigned values of −0.82 and 0.41 electron charge units, respectively, taken from the CVFF forcefield (Dauber-Osguthorpe et al. 1988). Radii of 1.4 Å and 1.0 Å have been assigned to these atoms, respectively, as in the case of charged oxygen and hydrogen atoms in PARSE. Alamethicin and the water inside the pore were assigned a dielectric constant of 2, whereas bulk water was assigned a dielectric constant of 80. The hydrocarbon region of the lipid bilayer was described as a slab of native width of 30 Å and a dielectric constant of 2. A lattice of 161^3 grid points and sequence of focusing runs of increasing resolution were employed to calculate ΔG_{elc} (Gilson et al. 1987). The calculation precision was estimated by comparing the values obtained for the last two resolutions, i.e. 1.5 versus 3 grid points per Å. ΔG_{elc} differences of up to ~0.5 kcal/mol were obtained.

$\Delta G_{np} = \gamma \Delta A + b$ was calculated as a linear function of the change in the water-accessible surface area of the channel upon association with the membrane (ΔA), with a surface tension coefficient of $\gamma = 0.0278$ kcal/(mol Å^2) and an intercept $b = -1.71$ kcal/mol (Sitkoff et al. 1996).

Estimation of ΔG_{lip} and ΔG_{imm}

ΔG_{lip} is the free energy penalty resulting from the interference of the solute with the conformational freedom of the lipid bilayer chains, and ΔG_{imm} is the free energy penalty resulting from the confinement of the external translational and rotational motion of the solute inside the membrane. In our previous studies with alamethicin monomers, we used values for ΔG_{lip} and ΔG_{imm} based on a statistical thermodynamic model of the lipid chains (Fattal and Ben-Shaul 1993; Ben-Shaul et al. 1996). The values obtained for ΔG_{lip} and ΔG_{imm} were 2.3 kcal/mol and 3.7 kcal/mol, respectively. They were obtained for the insertion of a hydrophobic cylinder-shape inclusion of radius 5 Å (corresponding to an α-helix) into a lipid bilayer with a hydrophobic width of 30 Å [corresponding to the width of a native POPC bilayer (White and Wimley 1999)]. The values we used in the present study are based on the above, but differ in two respects. First, these free energy terms depend on the effective radius of the cylindrical inclusion (R_p). Thus, the values obtained for the intact alamethicin channel ($R_p = 17$ Å) are significantly larger than those obtained for an alamethicin monomer ($R_p = 5$ Å). Secondly, ΔG_{lip} and ΔG_{imm} depend on the lipid bilayer width. As mentioned above, the values of Fattal and Ben-Shaul (1993) and Ben-Shaul et al. (1996) for ΔG_{lip} and ΔG_{imm} have been estimated for the insertion of inclusions into lipid bilayers of hydrophobic widths of 30 Å. The corresponding values for a lipid bilayer of width 22 Å have been estimated by May and Ben-Shaul (2000). We assumed a linear dependence of ΔG_{lip} and ΔG_{imm} on the membrane width, and extrapolated to obtain values for different lipid bilayer widths.

ΔG_{lip} and ΔG_{imm} (in kcal/mol) were calculated using the following formulas:

$$\Delta G_{lip} = 0.3 d_L + 9.53 \quad (3)$$

$$\Delta G_{imm} = 0.01 d_L + 4.73 \quad (4)$$

where d_L is the width of the hydrocarbon region of the bilayer in ångstrom. The values obtained for membrane widths of 23–26 Å are reported in Table 1.

Estimation of ΔG_{def}

Insertion of a solute into a lipid bilayer may result in a deformation of the lipid bilayer to match the width of the hydrocarbon region to the hydrophobic length of the solute, following the "mattress model" (Mouritsen and Bloom 1984). The deformation involves a free energy penalty, ΔG_{def}, resulting from the compression or expansion of the lipid chains. Based on the Fattal and Ben-Shaul (1993) model and the approximated channel dimensions mentioned above, one obtains the following equation:

$$\Delta G_{def} = 0.2636 d_L^2 - 15.579 d_L + 230.19 \quad (5)$$

This equation was used to estimate the ΔG_{def} values reported in Table 1.

Table 1 Free energy values for the different contributions to ΔG_{trans} (Eq. <equationcite>2</equationcite>) of different alamethicin channel conformations. The 11 conformations were produced by a 2 ns MD simulation in a POPC lipid bilayer (Tieleman et al. 1999). For each conformation, different channel–bilayer configurations were sampled, and continuum-solvent model calculations were employed to find the configuration of the most negative ΔG_{sol}. The other free energy contributions to ΔG_{trans} were added separately. The rows corresponding to structures that were found stable inside the bilayer are in bold characters

Structure[a]	Width (Å)[b]	ΔG_{elc} (kcal/mol)[c]	ΔG_{np} (kcal/mol)[d]	ΔG_{sol} (kcal/mol)[e]	ΔG_{imm} (kcal/mol)[f]	ΔG_{lip} (kcal/mol)[g]	ΔG_{def} (kcal/mol)[h]	ΔG_{trans} (kcal/mol)[i]
1 (initial)	**25**	**81.2**	**−117.2**	**−36.0**	**5.0**	**17.3**	**5.5**	**−8.2**
2	24	84.5	−106.2	−21.7	5.0	17.0	8.1	8.4
3	25	80.2	−107.6	−27.4	5.0	17.3	5.5	0.4
4	**23**	**59.4**	**−101.1**	**−41.7**	**5.0**	**16.7**	**11.3**	**−8.7**
5	24	81.7	−107.4	−25.7	5.0	17.0	8.1	4.4
6	24	74.7	−102.7	−28.0	5.0	17.0	8.1	2.1
7	26	101.7	−118.8	−17.1	5.1	17.7	3.3	9.0
8	24	92.3	−110.4	−18.1	5.0	17.0	8.1	12.0
9	23	80.5	−104.4	−23.9	5.0	16.7	11.3	9.1
10	**24**	**75.1**	**−110.9**	**−35.8**	**5.0**	**17.0**	**8.1**	**−5.7**
11 (final)	**24**	**65.5**	**−105.9**	**−40.4**	**5.0**	**17.0**	**8.1**	**−10.3**

[a]Structure index. The initial and final structures of the MD simulations are noted
[b]The width of the lipid bilayer where the orientation of most negative transfer free energy was found for each structure
[c]The electrostatic contribution to the solvation free energy
[d]The nonpolar contribution to the solvation free energy
[e]The solvation free energy
[f]The immobilization free energy
[g]The lipid perturbation free energy
[h]The membrane deformation free energy
[i]The water-to-membrane transfer free energy of the channel

Models of the alamethicin channel

The exact structure of the alamethicin channel has yet to be determined, although several models have been suggested (e.g. Fox and Richards 1982; Kerr et al. 1994; Breed et al. 1997a). In the present study we used models of the alamethicin channel suggested by Tieleman et al. (1999). Tieleman and co-workers used X-ray-based structures of alamethicin monomers in a 2 ns MD simulation in a POPC bilayer. These simulations produced numerous structures, 11 of which were used here. The 11 representative structures included the initial and end conformations of the simulation, and nine conformations which were taken every 200 ps during the simulation. The simulation snapshots, from which we took the channel structures, included the alamethicin channel (peptides and intra-pore water), POPC lipids, and bulk water. For the continuum solvent model calculations, we used only alamethicin and the water molecules inside the pore, while the lipid bilayer and bulk water were represented implicitly. We used the following manual procedure for exclusion of bulk water molecules. First, the surface area of the channel was calculated using InsightII (MSI, San Diego, USA). Then, bulk water molecules, which were positioned outside the boundaries of the surface area, were manually excluded.

Results

The free energy of channel insertion into the lipid bilayer

We considered a process for the formation of the alamethicin channel inside the lipid bilayer, in which the separate alamethicin monomers first acquire an ordered conformation, assemble to form an oligomeric channel, and only then insert into the lipid bilayer. Both ΔG_{assem} and ΔG_{con} are neglected in our calculations. Thus, the "total" free energy value we obtain corresponds to the transfer of the rigid channel from the aqueous solution to the lipid bilayer (ΔG_{trans}).

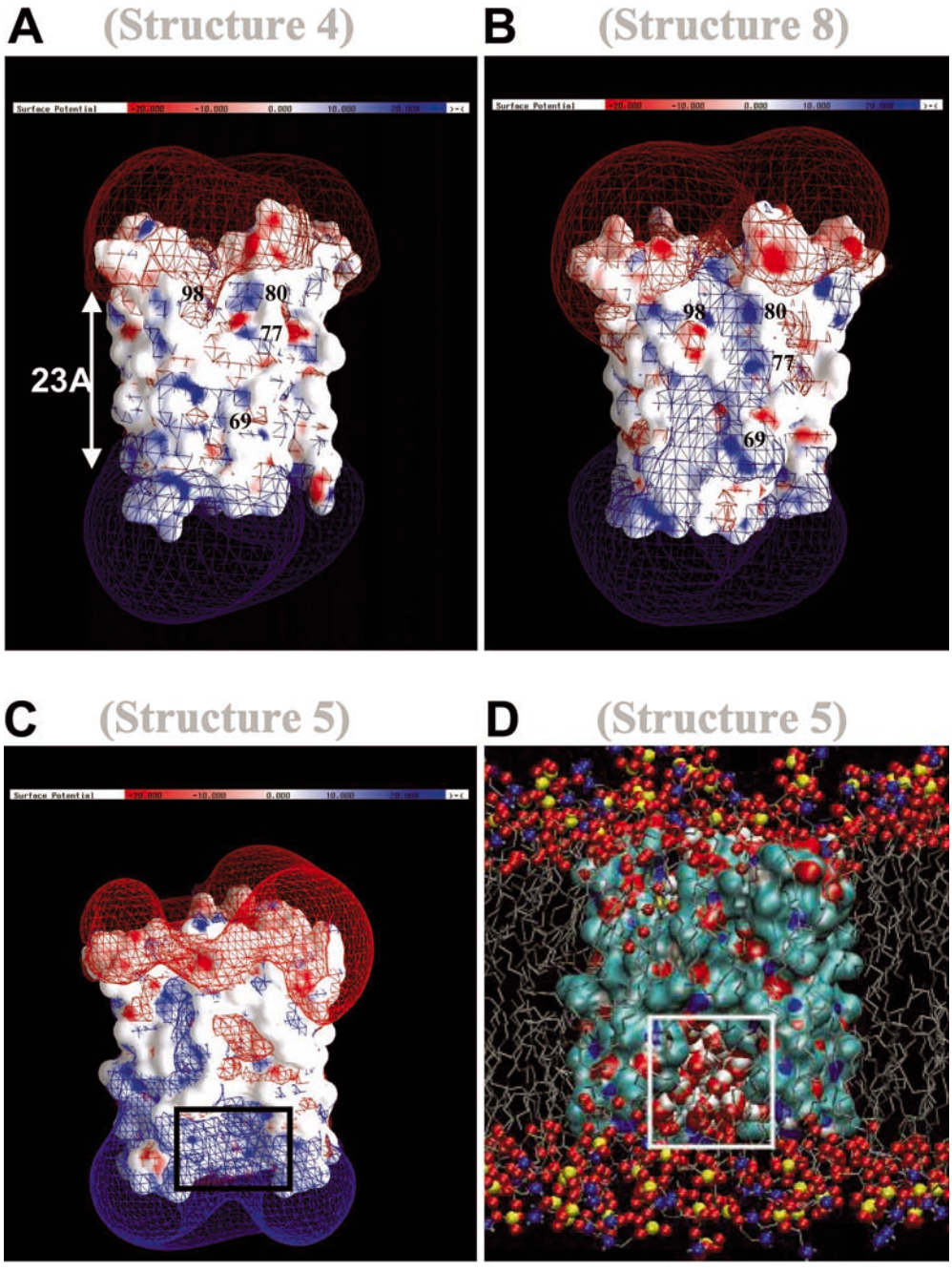
The calculated water-to-membrane transfer free energy values for all the structures are presented in Table 1. The results suggest that only four of the 11 structures used in the calculations are capable of partitioning into the lipid bilayer, with transfer free energies ranging from ∼−6 to ∼−10 kcal/mol. The rest of the structures appear to be unstable inside the lipid bilayer, as far as solvation is concerned, with transfer free energies ranging from ∼0 to ∼12 kcal/mol. As discussed below, the number of the membrane-stable structures may be even lower, owing to the neglect of the probably unfavorable (positive) contributions from the ΔG_{assem} and ΔG_{con} terms.

Channel insertion into the bilayer induces local membrane thinning

Our previous work demonstrated that the vertical insertion of alamethicin monomers into the lipid bilayer is likely to cause a deformation of the bilayer, which reduces its native width of 30 Å by ∼2 Å (Kessel et al. 2000a). In the present study we investigated the effect of the insertion of the oligomeric alamethicin channel on the membrane width in the vicinity of the oligomeric channel. As shown in Table 1, the most stable state of each of the 11 model structures involved significant membrane thinning. Membrane insertion of the four most stable channel structures was shown to induce a 5–7 Å thinning of the hydrocarbon region.

Electrostatic effects appear to determine channel stability inside the lipid bilayer

Our calculations (Table 1) show a significant variation (∼22 kcal/mol) in the water-to-membrane free energy of



transfer of the different channel structures. The free energy decomposition in Table 1 indicates that this variation results mainly from electrostatic effects. In order to determine the structural basis of these effects, we further investigated the structures, focusing on their electrostatic properties. Specifically, we calculated surface electrostatic potentials for each structure, and tried to identify the individual atoms and groups responsible for high-magnitude potentials.

The electrostatic analysis suggested two main causes for the instability of some of the channel structures inside the lipid bilayer. The first cause was uncompensated backbone hydrogen bonds in the region of the channel, which became exposed to the hydrocarbon of the lipid bilayer upon membrane insertion (Fig. 2A versus Fig. 2B; Fig. 3). The uncompensated hydrogen bonds resulted from distortions inside the alamethicin monomers. The second cause for the instability of some of the structures was the exposure of certain intra-pore water molecules to the surrounding lipid (Fig. 2C and 2D). This exposure was the result of disturbances in the interactions of some of the alamethicin monomers with each other.

The involvement of backbone hydrogen bonds in the membrane stability of peptides/protein segments has been demonstrated in other studies as well (White and

◄

Fig. 2A–D Electrostatic analysis of three of the channel model-structures. (**A**) Surface electrostatic potential maps of structure 4, which is associated with the second most negative value of ΔG_{trans}. (**B**) Surface electrostatic potential maps of structure 8, which is associated with the most positive value of ΔG_{trans}. The orientations in **A** and **B** are similar. The *arrow* in **A** indicates the length of the hydrophobic core of the channel. It is clear that the central region of structure 8, which is exposed to the hydrocarbon region of the lipid bilayer, is significantly more polar than that of structure 4 (relevant residues are noted). Analysis shows that the polarity results from uncompensated backbone hydrogen bonds. (**C**) A surface electrostatic potential map of structure 5, which is associated with a positive value of ΔG_{trans}. The large electrostatic potential at the lower region of the structure (marked by the *black rectangle*) results largely from intra-pore water molecules that are exposed to the surrounding environment. (**A**, **B** and **C**) The electrostatic potential (ϕ), calculated using DelPhi (Nicholls and Honig 1991), was color-coded and displayed on the molecular surface using GRASP (Nicholls et al. 1991). Negative potentials ($0 > \phi > -20\ kT/e$) are *red*, positive potentials ($0 < \phi < 20\ kT/e$) are *blue*, and neutral potentials are *white*. Three-dimensional equipotential contours are shown at $1\ kT/e$ (*blue mesh*) and $-1\ kT/e$ (*red mesh*). The peptides are shown with their N-termini pointing down and their more polar C-termini pointing up. (**D**) An all-atom model of structure 5 inside the lipid bilayer, taken from the MD simulations. The model is represented using the VMD program (Humphrey et al. 1996). The orientation of the channel in **D** is similar to its orientation in **C**. The water-accessible surface of the alamethicin channel is colored according to atom type (carbon atoms are *green*, nitrogen atoms are *blue*, and oxygen atoms are *red*). Intra-pore water molecules are described as space-filling models. The *gray lines* correspond to intra-lipid bonds involving a carbon atom, and the *red*, *blue*, and *yellow spheres* represent oxygen, nitrogen, and phosphorus atoms, respectively. The radius of the lipid atoms has been scaled down for clarity

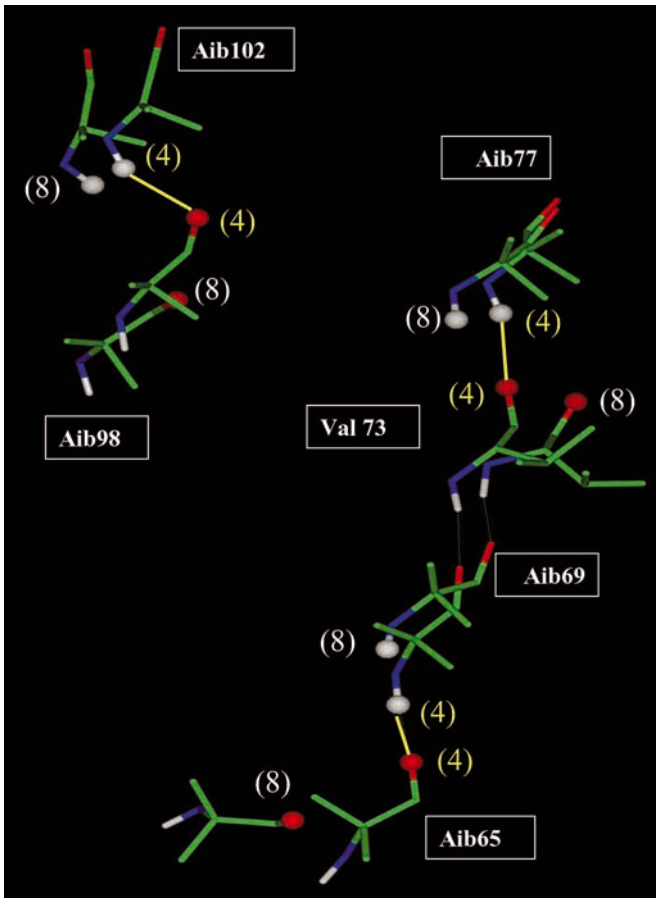


Fig. 3 Hydrogen bond differences between model structures 4 and 8 (Table 1, Fig. 2A and Fig. 2B) of the alamethicin channel. Structures 4 and 8 are noted *in parentheses*, in *yellow* and *white*, respectively. The figure displays residues Aib98, Aib102, Aib65, Aib69, Val73, and Aib77, which have been suggested by the electrostatic analysis of Fig. 2 to affect the channel's stability in the lipid bilayer. The bonds are presented as *sticks*. The backbone amide hydrogen and carbonyl oxygen atoms, which are involved in key hydrogen bonds, are presented as *balls* (InsightII, Accelrys, San Diego, Calif.). The two structures are superimposed according to the α-carbons of residues Aib65, Aib69, Val73, and Aib77, which reside on the same monomer. As clearly demonstrated, the backbone groups of residues Aib98, Aib102, Aib65, Aib69, Val73, and Aib77 form hydrogen bonds (presented as *yellow lines*) in structure 4, but not in structure 8

Wimley 1999; Kessel and Ben-Tal 2002). In the present study it was further supported by additional calculations, where we neutralized the electrostatic charges on backbone groups. The results, presented in Table 2, demonstrate the effect of charge neutralization of specific polar backbone groups in structures 4 and 8. The electrostatic analysis (Figs. 2 and 3) suggests that these groups affect the stability of the two structures inside the lipid bilayer. In accordance with Figs. 2 and 3, the neutralization of those groups significantly increased the stability of structure 8, whereas the stability of structure 4, which was relatively high to begin with, did not change much.

In addition to backbone polar groups, alamethicin contains two glutamine and one glutamic acid residues, both of which have polar sidechains. To investigate the relative effect of backbone versus sidechain polar groups on the stability of alamethicin channels inside the lipid bilayer, we neutralized the charges of either all backbone or all sidechain atoms, in the most membrane-stable (structure 11) and least membrane-stable (structure 8) structures. As Table 2 shows, regardless of the inherent stability of the structure in the membrane, the effect of backbone polar groups on the stabilization of alamethicin in the membrane environment is more than 10 times higher than that of the sidechain polar groups. This can be explained by the amphipathic nature of alamethicin, which results in a channel configuration where the sidechain of the polar residues of each monomer faces the aqueous pore. In such a configuration, the polar sidechains of these residues interact with, and are thus stabilized by, intra-pore water molecules. As a result, these polar groups do not significantly influence the stability of the channel inside the lipid bilayer. Conversely, at least some of the backbone groups are largely exposed to the lipid environment. This exposure involves a desolvation free-energy penalty, especially if the groups are not paired in hydrogen bonds. [It can be recalled that there is a free energy penalty of 2.1 kcal/mol for the transfer of a single satisfied backbone hydrogen bond (Ben-Tal et al. 1996; reviewed in White and Wimley 1999; Kessel and Ben-Tal

Table 2 Effect of protein polar groups on the water-to-membrane transfer free energy of different alamethicin channel structures. The role of the polar groups was determined by selectively neutralizing them and calculating ΔG_{trans}. For structures 4 and 8, the backbone carbonyl atoms of residues 65, 73, 98 and backbone amide atoms of residues 69, 77, 102 were neutralized (designated as $[cap]^{specific}$, respectively). These polar groups were suggested to be important in determining ΔG_{trans} (Figs. 2 and 3). For structures 8 and 11, either all the backbone (designated as $[cap]^{backbone}$) or sidechain (designated as $[cap]^{sidechain}$) polar groups, in all the monomers, were neutralized

Structure[a]	ΔG_{elc} (kcal/mol)[b]	ΔG_{np} (kcal/mol)[c]	ΔG_{sol} (kcal/mol)[d]	ΔG_{imm} (kcal/mol)[e]	ΔG_{lip} (kcal/mol)[f]	ΔG_{def} (kcal/mol)[g]	ΔG_{trans} (kcal/mol)[h]	$\Delta\Delta G_{trans}$ (kcal/mol)[i]
4	59.4	−101.1	−41.7	5.0	16.7	11.3	−8.7	
4 $[cap]^{specific}$	58.5	−101.1	−42.6	5.0	16.7	11.3	−9.6	−0.9
8	92.3	−110.4	−18.1	5.0	17.0	8.1	12.0	
8 $[cap]^{specific}$	82.4	−110.4	−28.0	5.0	17.0	8.1	2.1	−9.9
8 $[cap]^{backbone}$	20.3	−110.4	−90.1	5.0	17.0	8.1	−60	−62.1
8 $[cap]^{sidechain}$	88.6	−110.4	−21.8	5.0	17.0	8.1	8.3	−3.7
11	65.5	−105.9	−40.4	5.0	17.0	8.1	−10.3	
11 $[cap]^{backbone}$	15.0	−105.9	−90.9	5.0	17.0	8.1	−60.8	−50.5
11 $[cap]^{sidechain}$	62.4	−105.9	−43.5	5.0	17.0	8.1	−13.4	−3.1

[a]Structure index
[b]The electrostatic contribution to the solvation free energy
[c]The nonpolar contribution to the solvation free energy
[d]The solvation free energy
[e]The immobilization free energy
[f]The lipid perturbation free energy
[g]The membrane deformation free energy
[h]The water-to-membrane transfer free energy of the channel in a given structure
[i]The difference between the water-to-membrane transfer free energy of the capped and uncapped molecules

2002)]. Thus, the electrostatic neutralization of these groups in the entire protein results in a very marked stabilizing effect.

Inclusion of long-range coulombic interactions in the MD simulations have little effect on channel stability

Table 1 suggests that the MD simulations of Tieleman et al. (1999) were insensitive to the electrostatic effects which influence the stability of the alamethicin channel in the lipid bilayer. The insensitivity of the simulations may result, for example, from underestimation of the free energy penalty of the water-to-membrane transfer of polar groups in alamethicin. Such neglect may promote the breaking of hydrogen bonds, or the exposure of intra-pore water to the surrounding lipids, as was indeed observed in the structures we obtained from the MD simulation. To address this problem, we analyzed additional simulations, in which long-range electrostatic interactions were treated differently, as described below (Tieleman et al. 2002).

In all-atom simulations, the long-range coulombic interactions are treated by two cutoffs. Within the short-range cutoff, all interactions are calculated at every step. Between the short-range and the long-range cutoff, all interactions are calculated at every five or ten steps and assumed to be constant during this period; beyond the long-range cutoff, interactions are ignored. This, however, may lead to the neglect of electrostatic interactions that can be important for the stability and function of the protein. In the original MD simulation of Tieleman et al. (1999) a twin-range cutoff of 10/18 Å was used. In the new, modified simulations, we used two different long-range corrections for the electrostatic interactions. In the first, the region beyond 18 Å was considered as a high-dielectric continuum. In the second, Particle Mesh Ewald (Essmann et al. 1995) was used to calculate the electrostatic interactions beyond 10 Å. We chose representative structures from the simulations (four structures from the first, and 10 structures from the second), and assessed their stability inside the lipid bilayer by analyzing their electrostatic potentials. Such an analysis was found to be an efficient tool for this purpose in our previous work (Kessel et al. 2000a). The electrostatic maps of the structures (not shown) suggest that, overall, the inclusion of long-range electrostatic interactions did not improve the stability of the channel inside the lipid bilayer, and that the reaction field correction rendered it less stable than the original structures.

Increasing simulation time has little effect on channel stability

One of the major disadvantages of all-atom simulations is the harsh restriction on simulation time. That is, the simulation includes short-term dynamics, which may be insufficient to describe important processes in the system, such as key conformational changes in the protein. The original simulation of Tieleman et al. (1999) was 2 ns in length, which may not be enough time for relaxation of the system, and could be one of the causes for the apparent instability of the alamethicin channel structures. We investigated this by generating new structures using simulations 10 times longer (20 ns; Tieleman et al. 2002). The 20 representative structures chosen from the simulations were subjected to the same electrostatic analysis described above. The analysis, however, suggested that, on average, the length of the simulation did not change the stability of the channel inside the lipid bilayer (data not shown).

Minimization does not significantly change the stability of the channel structures

The channel structures used in our continuum-solvent calculations were taken directly as snapshots from the simulations of Tieleman et al. (1999). The simulation protocol did not include minimization of the snapshots, which could have reduced the conformational distortion effects mentioned above. To investigate this, we minimized the structures from the original simulations. The minimization was carried out in the presence of membrane lipids and bulk water, in the configuration of the corresponding simulation snapshot. The structures were then used in continuum-solvent model calculations to determine their membrane stability. The results (not shown) demonstrate that, in most cases, the minimization did not significantly change the water-to-membrane transfer free energy of the channel structures. Moreover, in no case did it render a membrane-unstable structure significantly stable.

Discussion

In this study we incorporated model structures of the alamethicin channel obtained from MD simulations, in continuum-solvent model calculations, to investigate long-term thermodynamic aspects of the channel in lipid bilayers. In the first part of the Discussion we refer to our main results, and in the second part we discuss the ramifications of these results for the use of continuum versus all-atom models in protein/peptide–membrane simulations.

Thermodynamics of channel insertion into the membrane

We have used continuum-solvent model calculations to determine the membrane stability of 11 representative structures of the alamethicin channel, produced by MD simulations in POPC bilayers. The results (Table 1) demonstrate remarkable differences, ranging from ~ -10 to ~ 12 kcal/mol, in the value of the water-to-membrane transfer free energies of the structures. To our knowledge, the free energy of alamethicin channel insertion into lipid bilayers has yet to be experimentally or theoretically determined. Moreover, the alamethicin channel has been found to shift between different conduction states, which are characterized by different numbers of monomers (Sansom 1993). Channel structures of different monomeric composition should insert into the membrane with different free energy values.

Our calculations suggest that, of the 11 structures we investigated, seven were unstable inside the lipid bilayer. As explained in Methods, our calculations neglected the ΔG_{con} and ΔG_{assem} contributions to ΔG_{tot}. Since both contributions are probably unfavorable (i.e. have positive values), the values of ΔG_{tot} for the 11 structures may be less favorable than the ΔG_{trans} values in Table 1; that is, the fraction of membrane-stable structures may be even lower.

We analyzed the different channel structures to determine the factors affecting the stability of the channel inside membranes. The analysis indicated that the main differences in the free energy of membrane association of the different structures arise from electrostatic effects, which result from exposure of either uncompensated hydrogen bonds inside the monomers or intra-pore water molecules to the hydrocarbon core of the lipid bilayer (Fig. 2). These are the respective consequences of disturbances in the α-helical conformation of the monomers and in monomer–monomer interactions. Our previous studies demonstrated the significance of electrostatic effects in determining the stability of alamethicin monomers inside lipid bilayers (e.g. Kessel et al. 2000a, 2000b). Moreover, the strong involvement of the peptide backbone groups in these effects is stressed by both theoretical and experimental studies (e.g. White and Wimley 1999; Kessel and Ben-Tal 2002).

The hydrophobic lengths of the 11 channel structures are significantly shorter than 30 Å, the width of the hydrocarbon region of a native (i.e. unperturbed) lipid bilayer (White and Wimley 1999). For example, the hydrophobic length of structure 4, which was found to be stable in the membrane, is only 23 Å (Fig. 2A). A large electrostatic-desolvation free-energy penalty would be associated with the transfer of the polar groups at the boundaries of the channel's hydrophobic region from the aqueous phase into the hydrocarbon region of the membrane (Kessel and Ben-Tal 2002). The calculations reported here show that an alternative, involving local thinning at the membrane–channel interaction region to match the width of the hydrocarbon region with the hydrophobic length of the channel, was found to be energetically favorable (Table 1). Membrane insertion of each of the 11 channel structures into the membrane induced a significant membrane thinning of 4–7 Å. The four structures that were found to be stable in the membrane were at the high end and induced membrane thinning of 5–7 Å. Owing to the model nature of these structures, it is impossible at this point to tell if similar membrane thinning may be observed experimentally.

It should be noted that in the MD simulations the insertion of the channel into the POPC bilayer induced an average thinning of the bilayer of only 2 Å (Tieleman et al. 1998). As discussed in detail below, the simulations appear to be less sensitive than the continuum-solvent model calculations to the exposure of polar groups in the channel to the hydrocarbon core of the lipid bilayer. We suggest that those differences are the main reason for the overall discrepancy between the results obtained by the two methods. This is probably also true for the discrepancy about the degree of thinning of the lipid bilayer; the high electrostatic free energy penalty resulting from this exposure is the cause of the dramatic defor-

mation of the membrane, as seen in the continuum-solvent model calculations.

Mean-field versus all-atom approaches in the study of channel–membrane interactions

The continuum solvent model calculations presented here appear to be in discrepancy with the MD simulations of Tieleman et al. (1999). Specifically, our calculations found most of the representative structures from the simulation to be unstable in the lipid bilayer, with free energies of transfer of up to 12 kcal/mol. Although we could not unequivocally determine the reasons for the discrepancy, several options come to mind and we discuss them in light of the basic differences between the two approaches.

The approximations made by the continuum-solvent model have been previously discussed in detail (Ben-Tal and Honig 1996; Kessel et al. 2000a, 2000b, 2001; Kessel and Ben-Tal 2002). The main uncertainty of the model results from the complete neglect of the polar headgroup region of the bilayer, which may obscure both general and specific interactions between alamethicin and this region. Polar lipid groups have been shown to interact with different proteins and peptides, and mediate their binding to membranes. Some of the best-studied cases involve positively charged proteins/peptides, such as MARCKS, that adsorb onto the membrane surface and interact coulombically with negatively charged lipids (Murray et al. 2002)

Alamethicin monomers can also adsorb onto the surface of the membrane. However, since both alamethicin and the lipid polar headgroups are uncharged, the coulombic interactions between them should be considerably less significant. Moreover, the adsorption process is not considered in our calculations, whereas in real life the channel is probably formed inside the membrane from monomers that were separately inserted; in the current study we consider the insertion of the intact channel into the bilayer. This process does not include the adsorption of the single peptide onto the membrane, and the only interactions of alamethicin with the polar headgroups that exist in this system are likely to involve the channel's uncharged termini only. Unfortunately, at this point, we cannot determine the magnitude of stabilization of the channel by its surrounding lipids. However, we believe that this stabilization is of minor importance. Indeed, our previous studies with monomeric alamethicin indicate that the alamethicin–headgroups interactions have a minor effect on the free energy of association of the peptide with the membrane (Kessel et al. 2000a). This should be especially true for the oligomeric channel, in which monomer–monomer interactions replace some of the monomer–membrane interactions.

Still, peptide–lipid interactions may be important for the stabilization of the channel in specific cases, where polar groups all over the channel are transiently exposed to the low dielectric environment of the channel. We discuss this issue in detail below.

Another uncertainty in the continuum-solvent model involves the neglect of energy contributions resulting from conformational changes in the protein upon its association with the lipid bilayer (ΔG_{con}). Such contributions may, at least partially, compensate for the unfavorable solvation free energy (ΔG_{sol}) of some of the simulation-produced structures. We used the data from the MD simulations to investigate this possibility. Although ΔG_{con} could not be obtained from the simulations, we used the internal potentials of the different channel structures (peptides plus pore water) to see if there was any correlation between the internal potentials and the free energy from the continuum-solvent model calculations. The internal energies were calculated using the potential function of GROMACS (Berendsen et al. 1995). The results demonstrated no correlation between the two energy terms (data not shown), suggesting that they do not balance each other. It should be mentioned that, in addition to the internal energy, ΔG_{con} should also include free energy contributions that result from changes in the conformational entropy of the channel upon binding to the bilayer. Unfortunately, such contributions are difficult to calculate, and were neglected.

Other uncertainties of the continuum-solvent approach result from the way we treated the water molecules inside the pore. First, PARSE does not include radii and partial charges for water molecules, and we used the corresponding values from the CVFF forcefield (Dauber-Osguthorpe et al. 1988). This might have influenced the calculation of the solvation free energy values, which depend strongly on the choice of the set of atomic partial charges and radii. To assess the magnitude of this effect, we calculated the water-to-membrane transfer free energy of all the alamethicin channel structures with their intra-pore water molecules electrostatically neutralized. The calculations (data not shown) demonstrated that, in most cases, this dramatic change in the assigned charges of the water molecules changed ΔG_{trans} by $\sim$5 kcal/mol. This value may be regarded as an upper bound for the error resulting from assignment of wrong charges to water molecules; the real value of the error is obviously much lower. In any case, the calculations showed that, in most of the channel structures, even the upper bound value for the error did not change the inherent propensity of the structure to either remain in the aqueous solution or to partition into the lipid bilayer.

The second uncertainty related to the treatment of intra-pore water involves their definition. The aqueous environment inside the pore of the ion channel can either be described implicitly, as a high-dielectric continuum, or explicitly. An implicit description of the intra-pore environment was used in our study of gramicidin (Bransburg-Zabary et al. 2002), where the available experimental structures did not contain intra-pore water molecules. However, this description is problematic, since the dielectric properties of the pore have not yet

been characterized, and are probably not homogenous. We therefore chose to describe the water inside the channel pore explicitly. The original simulation snapshots used for our calculations included the protein, intra-pore water, membrane lipids, and bulk water. The explicit treatment of the intra-pore water required their distinction from bulk water molecules. This was done manually, and thus less accurately. That is, some water molecules were included as intra-pore water in some of the structures, while they were excluded as bulk water in the other structures. We believe that this does not pose a serious problem for two reasons. First, as mentioned above, our calculations suggest that the contribution of intra-pore water molecules to the total free energy of association of the channel with the lipid bilayer is limited, and does not exceed ~5 kcal/mol. Second, all the excluded water molecules were positioned at the channel termini, where solvent screening effects are dominant.

The third uncertainty involves the neglect of the free energy of desolvation of intra-pore water upon the association of the channel with the lipid bilayer. Our model took into account the desolvation free energy of the channel by considering its contact area with bulk water or membrane lipids. The desolvation of intra-pore water was not taken into account, since these molecules do not contribute to the contact area of the channel.

The continuum-solvent model assumes the rigidity of the water–membrane interface, and exclusion of alamethicin's polar groups from the low-dielectric environment of the lipid bilayer is made possible only by overall deformation of the lipid bilayer. The deformation is energetically costly, and can therefore take place only up to a certain degree. Beyond that, the lipid bilayer will not deform, and the lipid exposure of protein polar groups will significantly raise the free energy of the system. In reality, however, the water–membrane interface is dynamic. Fluctuations of membrane lipids may stabilize polar groups at the termini of alamethicin by forming local deformations that facilitate the surrounding peptide polar groups (or exposed intra-pore water molecules) by the polar lipid heads. The local deformations are likely to involve a lower free energy penalty than the overall deformation of the lipid bilayer, and should therefore lower ΔG_{trans}. However, lipid fluctuations leading to increased burial of peptide polar groups inside the membrane should also take place. It is difficult to determine which fluctuations are more dominant. Thus, we choose to consider the average propensity of the membrane to deform.

This choice is supported by the analysis of model structure 5 of the channel. This structure appears to be unstable inside the membrane (Table 1), and panels C and D of Fig. 2 demonstrate that this is due to the exposure of certain intra-pore water molecules in the structure to the hydrocarbon region of the lipids. The continuum-solvent model calculations suggest that, upon membrane insertion, those water molecules become exposed to the hydrocarbon region of the lipid bilayer, despite the overall deformation of the membrane. Again, it may be argued that local fluctuations in the lipid bilayer, which are not considered by the continuum-solvent model, enable certain polar lipid headgroups to mask the intra-pore water from the hydrophobic core of the membrane. However, panel D of Fig. 2, which presents structure 5 within the context of the all-atom lipid bilayer, suggests otherwise: although certain membrane lipids do penetrate inside the hydrocarbon region of the membrane, most of the intra-pore water molecules in the alamethicin channel do not appear to be masked by any polar groups in the lipid bilayer. It should be noted that the channel–membrane configuration presented in Fig. 2D is a single snapshot of structure 5, taken directly from the MD simulations. Local lipid rearrangements can, at least theoretically, occur immediately before or after this snapshot was taken. However, the relatively slow nature of lipid dynamics prevents significant fluctuations in the membrane from taking place in the narrow time frame between the snapshots (i.e. 200 ps).

A similar argument can also be made for bulk water. That is, local membrane fluctuations may allow limited penetration of individual water molecules to the hydrocarbon region of the membrane, and a subsequent masking of the channel's polar groups by these water molecules. Again, inspection of the snapshots from the simulation did not reveal that bulk water penetrated into the hydrocarbon core of the bilayer to the extent that is was able to efficiently mask exposed polar groups in the channel (data not shown).

As explained in our previous papers (e.g. Kessel et al. 2000a), we calculate ΔG_{np} based on the water-accessible surface area of the solute. Although this type of calculation is conventional in continuum-solvent model studies, the recent work of Pitera and van Gunsteren (2001) suggests that it may not be as accurate as previously thought. The authors suggest that the atoms at the interior of the solute, while not in direct contact with the solvent, still make favorable van der Waals interactions with solvent atoms. The calculation of ΔG_{np} based on the surface area of the solute (e.g. the channel) alone neglects these interactions, and may therefore be inaccurate. This claim was supported by their calculations, showing that for ubiquitin the neglect of the atoms at the protein interior involved a ~45 kcal/mol difference in the solvation free energy, compared to the "full" protein. While we accept the general notion that the neglect of the atoms at the protein interior is likely to change ΔG_{np}, we believe that, in our system, the actual value of the neglected free energy is considerably lower than suggested. This is based on the fact that, in the calculations of Pitera and van Gunsteren (2001), the solute (ubiquitin) was transferred from the vacuum, in which solute–solvent interactions are nonexistent, to water, in which such interactions exist. In our system, the alamethicin channel was transferred from one solvent (water) to another (the lipid bilayer). The magnitude of channel–solvent interactions in the transfer between the two media should roughly be the

same, as evident from the low magnitude of the surface tension coefficient associated with water-to-liquid alkane transfer (Sitkoff et al. 1996), which makes the difference in ΔG_{np} between the "full" and "hollow" forms of the protein smaller.

Finally, our continuum-solvent results may be the consequence of our choice of models. First, we used only 11 structures, taken from the MD simulation at 200 ps intervals. Our choice to use a small, representative number of structures resulted primarily from computational cost considerations. Continuum-solvent model calculations are computationally inexpensive as compared to all-atom calculations. However, when used on an oligomeric (hexameric) structure (which also includes numerous water molecules) and sampling of many channel–membrane configurations for each structure, the calculations are considerably long. Still, it can be argued that 11 structures are statistically insignificant, and that additional structures, taken in-between the 200 ps intervals, could demonstrate better electrostatic stability. Moreover, better structures could have been chosen, for example structures in which intra-pore water are less exposed to the lipid environment. While we generally agree that this could be the case, we like to note that the mere existence of structures along the entire simulation, which appear to be incompatible with the membrane environment, as judged by the continuum-solvent model calculations, demonstrates our point; that is, that there is a discrepancy between the two methods.

Second, we carried out our calculations on a hexameric configuration of the channel, whereas studies suggest that the alamethicin channel can exist in different oligomeric states, with the number of monomers estimated to range between 4 and 11. Different oligomeric states may differ in their compatibility with the membrane environment. We chose a hexameric model of the channel because it was the only available model at the time. However, regardless of the relative stability of the different channel configurations, by the MD simulation each of them, including the hexameric state, should be stable inside the lipid bilayer.

Alternatively, the discrepancy between the continuum-solvent model calculations and the MD simulations may result from some of the uncertainties associated with the latter. MD simulations of peptide–membrane systems operate on a nanosecond scale. This may be sufficient for describing short-term processes, such as the formation of hydrogen bonds, or the alignment of intra-pore water molecules, but may not be long enough for the description of long-term processes, such as the overall changes in protein conformation needed for its stabilization inside the lipid bilayer. We used channel structures, which were originally constructed to be stable in lipid bilayers, and the continuum solvent model calculations showed that the initial conformation was indeed stable in the membrane. Therefore, the simulations in this case should, at least in theory, be able to describe the adaptation of the channel to the lipid environment. Still, longer simulations might create more stable structures of the channel. However, analysis of the channel structures suggests that the instability of at least some of the structures may result from moderate conformational changes, which may be within the nanosecond range, and should therefore be considered by the simulation. For example, the trace root mean square deviation (RMSD) of the most stable (structure 11 in Table 1) and the least stable (structure 8 in Table 1) structures in the lipid bilayer is only 1.2 Å, although their membrane association free energies differ by 22.3 kcal/mol. This suggests that the membrane stability of the channel may be influenced by factors other than the simulation time. Indeed, multiplication of the simulation time by a factor of 10 (i.e. to 20 ns) did not improve channel stability, as implied by electrostatic analysis of the structures produced by the simulation.

The discrepancy between the continuum-solvent model calculations and the MD simulations could result from the consideration of the electrostatic contributions to the solvation free energy. For example, the apparent insensitivity of the MD simulations to the membrane stability of the channel could originate from the distance cutoff used for calculating the electrostatic interactions in the system. That is, the use of a cutoff may have led to an underestimation of the electrostatic penalty for desolvation. We applied long-range electrostatic corrections to account for the possible neglect. Again, electrostatic analysis of the structures generated using this correction suggests that these modifications did not improve the membrane stability of the channel (data not shown).

Other reasons for the discrepancy related to the electrostatic free energy could involve the parameters on which the calculation is based. The atomic radii and partial charges used in the continuum-solvent model calculations were taken from the PARSE parameter set (Sitkoff et al. 1994, 1996). Continuum-solvent model calculations using PARSE reproduced the experimental alkane-to-water transfer free energies of various small molecules, comprised of the chemical groups that constitute proteins, with an average absolute error of 0.21 kcal/mol and a maximum error of 1.15 kcal/mol (Sitkoff et al. 1996). Recent studies demonstrated the potency of continuum-solvent model calculations using PARSE in reproducing various empirical data on peptide–membrane systems in general (Ben-Tal et al. 1996; Bransburg-Zabary et al. 2002) and on alamethicin–membrane interactions in particular (Kessel et al. 2000a, 2000b).

In contrast, to the best of our knowledge, the forcefield used in the MD simulations was not parameterized to reproduce solvation free energies. [Indeed, studies based on MD simulations sometimes use continuum-solvent model calculations to address thermodynamic aspects of protein–membrane interactions (e.g. Bernèche et al. 1998).] Thus, it is possible that the electrostatic penalty due to the transfer of polar groups from the aqueous phase into the

hydrocarbon region of the lipid bilayer is underestimated in the MD simulations. Systematic continuum-solvent model calculations of both vacuum-to-water and alkane-to-water solvation free energies of small molecules using atomic radii and partial charges from commonly used forcefields demonstrated that the magnitude of the electrostatic free energy contribution is smaller than the experimentally derived estimate (Sitkoff et al. 1994, 1996).

Conclusions

Continuum-solvent model and MD calculations are powerful tools for simulating molecular systems. Both are commonly used to study protein–membrane interactions, but the results presented above show an apparent discrepancy between them in determination of protein stability inside membranes. Two possibilities for resolving the discrepancy come to mind:

1. The results of both the continuum-solvent model calculations and the MD simulations are accurate and the large differences in the electrostatic solvation free energy between the different alamethicin channel structures are balanced by other energy terms (e.g. ΔG_{con}). This implies a very rapid energy transfer between degrees of freedom (e.g. between ΔG_{sol} and ΔG_{con}).
2. Alternatively, the problem could be due to inaccuracies in one of the methods or in both. We cannot, at this time, point to the exact cause for the discrepancy, but Table 1 suggests that it results mainly from the two methods' different treatment of electrostatic desolvation effects. More data are needed for a complete comparison between the methods. Such data are likely to be achieved more efficiently by using simpler systems based on single peptides or smaller molecules.

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References

Banerjee U, Chan SI (1983) Structure of alamethicin in solution: nuclear magnetic resonance relaxation studies. Biochemistry 22:3709–3713

Barranger-Mathys M, Cafiso DS (1994) Collisions between helical peptides in membranes monitored using electron paramagnetic resonance: evidence that alamethicin is monomeric in the absence of a membrane potential. Biophys J 67:172–176

Baumann G, Mueller P (1974) A molecular model of membrane excitability. J Supramol Struct 2:538–557

Ben-Shaul A, Ben-Tal N, Honig B (1996) Statistical thermodynamic analysis of peptide and protein insertion into lipid membranes. Biophys J 71:130–137

Ben-Tal N, Honig B (1996) Helix-helix interactions in lipid bilayers. Biophys J 71:3046–3050

Ben-Tal N, Ben-Shaul A, Nicholls A, Honig B (1996) Free-energy determinants of alpha-helix insertion into lipid bilayers. Biophys J 70:1803–1812

Berendsen HJC, van der Spoel D, van Drunen R (1995) GROMACS: a message-passing parallel molecular dynamics implementation. Comput Phys Commun 95:43–56

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

Boheim G (1974) Statistical analysis of alamethicin channels in black lipid membranes. J Membr Biol 19:277–303

Bransburg-Zabary S, Kessel A, Gutman M, Ben-Tal N (2002) Stability of an ion channel in lipid bilayers: implicit solvent model calculations with gramicidin. Biochemistry 41: 6946–6954

Breed J, Sankararamakrishnan R, Kerr ID, Sansom MS (1996) Molecular dynamics simulations of water within models of ion channels. Biophys J 70:1643–1661

Breed J, Biggin PC, Kerr ID, Smart OS, Sansom MS (1997a) Alamethicin channels: modelling via restrained molecular dynamics simulations. Biochim Biophys Acta 1325:235–249

Breed J, Kerr ID, Molle G, Duclohier H, Sansom MS (1997b) Ion channel stability and hydrogen bonding. Molecular modelling of channels formed by synthetic alamethicin analogues. Biochim Biophys Acta 1330:103–109

Brooks CL 3rd (1998) Simulations of protein folding and unfolding. Curr Opin Struct Biol 8:222–226

Cafiso D (1994) Alamethicin: a peptide model for voltage gating and protein-membrane interactions. Annu Rev Biophys Biomol Struct 23:141–165

Dauber-Osguthorpe P, Roberts VA, Osguthorpe DJ, Wolff J, Genest M, Hagler AT (1988) Structure and energetics of ligand binding to proteins: *Escherichia coli* dihydrofolate reductase-trimethoprim, a drug-receptor system. Proteins 4:31–47

Engelman DM, Steitz TA (1981) The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. Cell 23:411–422

Esposito G, Carver JA, Boyd J, Campbell ID (1987) High-resolution ^{1}H NMR study of the solution structure of alamethicin. Biochemistry 26:1043–1050

Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG (1995) A smooth particle mesh Ewald method. J Chem Phys 103:8577–8593

Fattal DR, Ben-Shaul A (1993) A molecular model for lipid-protein interaction in membranes: the role of hydrophobic mismatch. Biophys J 65:1795–1809

Fox RO Jr, Richards FM (1982) A voltage-gated ion channel model inferred from the crystal structure of alamethicin at 1.5-A resolution. Nature 300:325–330

Gilson MK, Sharp KA, Honig B (1987) Calculating the electrostatic potential of molecules in solution: method and error assessment. J Comput Chem 9:327–335

Gordon LG, Haydon DA (1975) Potential-dependent conductances in lipid membranes containing alamethicin. Philos Trans R Soc Lond B Biol Sci 270:433–447

Guerois R, Serrano L (2001) Protein design based on folding models. Curr Opin Struct Biol 11:101–106

Humphrey W, Dalke A, Schulten K (1996) VMD: visual molecular dynamics. J Mol Graphics 14:33–38

Jacobs RE, White SH (1989) The nature of the hydrophobic binding of small peptides at the bilayer interface: implications for the insertion of transbilayer helices. Biochemistry 28:3421–3437

Kerr ID, Sankararamakrishnan R, Smart OS, Sansom MS (1994) Parallel helix bundles and ion channels: molecular modeling via simulated annealing and restrained molecular dynamics. Biophys J 67:1501–1515

Kessel A, Ben-Tal N (2002) Free energy determinants of peptide association with lipid bilayers. In: Simon S, McIntosh T (eds) Current topics in membranes, vol 52: peptide-lipid interactions. Academic Press, San Diego, pp 205–253

Kessel A, Cafiso DS, Ben-Tal N (2000a) Continuum solvent model calculations of alamethicin-membrane interactions: thermodynamic aspects. Biophys J 78:571–583

Kessel A, Schulten K, Ben-Tal N (2000b) Calculations suggest a pathway for the transmembrane migration of a hydrophobic peptide. Biophys J 79:2322–2330

Kessel A, Musafia B, Ben-Tal N (2001) Continuum solvent model studies of the interactions of an anticonvulsant drug with lipid bilayers. Biophys J 80:2535–2545

May S, Ben-Shaul A (2000) A molecular model for lipid-mediated interaction between proteins in membranes. Phys Chem Chem Phys 2:4494–4502

Milik M, Skolnick J (1993) Insertion of peptide chains into lipid membranes: an off-lattice Monte Carlo dynamics model. Proteins 15:10–25

Mouritsen OG, Bloom M (1984) Mattress model of lipid-protein interactions in membranes. Biophys J 46:141–153

Murray D, Arbuzova A, Honig B, McLaughlin S (2002) The role of electrostatic and nonpolar interactions in the association of peripheral proteins with membranes. In: Simon S, McIntosh T (eds) Current topics in membranes, vol 52: peptide-lipid interactions. Academic Press, San Diego, pp 271–302

Nicholls A, Honig B (1991) A rapid finite difference algorithm, utilizing successive over-relaxation to solve the Poisson-Boltzmann equation. J Comput Chem 12:435–445

Nicholls A, Sharp KA, Honig B (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. Proteins 11:281–296

North CL, Barranger-Mathys M, Cafiso DS (1995) Membrane orientation of the N-terminal segment of alamethicin determined by solid-state ^{15}N NMR. Biophys J 69:2392–2397

Pitera JW, van Gunsteren WF (2001) The importance of solute-solvent van der Waals interactions with interior atoms of biopolymers. J Am Chem Soc 123:3163–3164

Sansom MS (1993) Structure and function of channel-forming peptaibols. Q Rev Biophys 26:365–421

Sansom MS (1998) Models and simulations of ion channels and related membrane proteins. Curr Opin Struct Biol 8:237–244

Sitkoff D, Sharp K, Honig B (1994) Acurate calculations of hydration free energies using macroscopic solvent models. J Phys Chem 98:1978–1988

Sitkoff D, Ben-Tal N, Honig B (1996) Calculation of alkane to water solvation free energies using continuum solvent models. J Phys Chem 100:2744–2752

Tieleman DP, Sansom MSP (2001) Molecular dynamics simulations of antimicrobial peptides: from membrane binding to transmembrane channels. Int J Quantum Chem 83:166–179

Tieleman DP, Forrest LR, Sansom MS, Berendsen HJ (1998) Lipid properties and the orientation of aromatic residues in OmpF, influenza M2, and alamethicin systems: molecular dynamics simulations. Biochemistry 37:17554–17561

Tieleman DP, Berendsen HJ, Sansom MS (1999) An alamethicin channel in a lipid bilayer: molecular dynamics simulations. Biophys J 76:1757–1769

Tieleman DP, Hess B, Sansom MSP (2002) Analysis and evaluation of channel models: simulations of alamethicin. Biophys J 83:2393–2407

White SH, Wimley WC (1999) Membrane protein folding and stability: physical principles. Annu Rev Biophys Biomol Struct 28:319–365

Yee AA, O'Neil JD (1992) Uniform ^{15}N labeling of a fungal peptide: the structure and dynamics of an alamethicin by ^{15}N and ^{1}H NMR spectroscopy. Biochemistry 31:3135–3143