Effects of Electric Field on Alamethicin Bound at the Lipid-Water Interface: A Molecular Mechanics Study

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ABSTRACT A systematic molecular mechanics study of the alamethicin molecule was made to determine a set of low-energy conformers in vacuo and in aqueous environment. The behavior of these conformers was investigated at the phase boundary which was modeled as a plane dividing two compartments with solvation properties of water and octanol with a constant electric field applied normal to the boundary. The calculations were performed with a molecular mechanics program for calculation of stable conformations at the phase boundary utilizing the Empiric Conformational Energy Program for Peptides force field and the Hopfinger-Scheraga solvation model. 371 minimum energy conformers of alamethicin, determined in vacuo with the build-up procedure, were used as starting conformations for energy minimization in aqueous environment and at the phase boundary. Only 49 interphase-bound structures were within 12 kcal/mol of the minima which was found. No helical structures having values close to the canonical parameters for an α- or 3_{10}-helix were found despite the presence of eight α-methylalanine residues which favor the formation of these helices; four helix-like structures were found, having all negative ϕ, ψ values. All the helical conformers have very high energies in water (~14 kcal/mol), but are quite stable at the phase boundary (3.7–6.8 kcal/mol above the lowest minima found). The implications of these results for proposed mechanisms for membrane-binding and voltage-dependent gating are considered.

INTRODUCTION

Alamethicin is a relatively simple 20-residue peptide which is able to form voltage-gated channels in lipid bilayers. For more then two decades, the enigmatic gating mechanism has been a real challenge for membrane biophysicists; researchers have investigated this simplified model system, comprised of just a few low-molecular weight, precisely identified components. And yet, despite the intensive efforts of many highly qualified investigators, the first step, the process of binding of alamethicin to lipids, is not understood in detail, let alone the mechanism of alamethicin-induced, voltage-dependent changes in membrane conductance.

The peptide-lipid and peptide-peptide interactions are processes with a very complex stoichiometry. The affinity of the alamethicin to the lipid phase increases as the concentration of membrane-bound peptide rises; this cooperativity is thought to be the effect of aggregation of membrane-bound peptide (Schwarz and Savko, 1982; Schwarz et al., 1986, 1987), though a recent investigation failed to discover any significant amount of aggregates of spin-labeled alamethicin analogs in phospholipid vesicles (Archer et al., 1991) and thylacoids (Wille et al., 1989). On the other hand, the conductivity of alamethicin-treated membranes increase with a dependence on the 4th to 10th power of the concentration (Boheim and Kolb, 1978; Archer and Cafiso, 1991), so that functional channels must be aggregates. Probably, only a small fraction of lipid-bound alamethicin forms channels (Archer et al., 1991) and study of macroscopic properties may, in fact, obscure the mechanistic details of channel behavior.

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It is commonly assumed that the alamethicin channel in its conducting state is an aggregate of several molecules in helical conformations. All models proposed for the explanation of the alamethicin-induced gating effects suggest such a monomer structure as part of an open, ion-conducting channel (Marshall and Beusen, 1992; Sansom, 1991). Helical, or predominantly helical structures, were found for alamethicin in the crystal (Fox and Richards, 1982), in different organic solvents (Kelsh et al., 1992; Esposito et al., 1987; Banerjee and Chan, 1983) and in lipid-bound form (Cascio and Wallace, 1988; Vogel, 1987). These observations are also consistent with the data on the dipole moment of alamethicin in nonpolar solvents (Schwarz and Savko, 1982; Yantorno et al., 1982). Molecular mechanics methods were also used to investigate the conformational and aggregational properties of different helical forms of alamethicin and some related peptides (Sansom et al., 1991; Pullman, 1991; Furois-Corbin and Pullman, 1988). An important result was obtained (Furois-Corbin and Pullman, 1988); namely, the overall energy of interaction of a pair of parallel, helical amino-terminal fragments of alamethicin was shown to be negative despite strong electrostatic repulsion. Formerly, Edmonds has shown that electrostatic interactions of a pair of helices are comparable with kT (Edmonds, 1985).

In this paper, we report the results of a systematic study of the alamethicin molecule by molecular mechanics in which sets of low-energy conformers were determined both in vacuo and in an aqueous environment using a continuum solvation model (Hodes et al., 1979; Hopfinger and Battershell, 1976). The use of such a solvation model to calculate behavior at a phase boundary had been previously validated (Galaktionov et al., 1988) by calculation of transfer energies of a set of peptides from water to the lipophilic phase boundary; the calculated values correlated excellently with exper-

imental data on surface activity. The behavior of these conformers of the alamethicin molecule at the phase boundary, "water-lipophilic phase," with an electric field applied across the boundary was also determined. The variety of models proposed for the mechanism of voltage gating by alamethicin were examined based on their consistency with the results obtained from these calculations.

METHODS

Calculations in vacuo

Standard molecular mechanics based on the Empiric Conformational Energy Program for Peptides (ECEPP) force field and rigid valence geometry (Dunfield et al., 1978; Momany et al., 1975; Nemethy et al., 1983) was used for intramolecular energy calculations and elucidation of the set of stable alamethicin conformers. Energy minimization used the Fletcher-Powell-Davidon algorithm (Himmelblau, 1972).

The energy calculation pattern based on a build-up procedure is schematically presented on Fig. 1. We started with calculation of two decapeptides: 1–10 and 11–20. Sequential steps of this procedure involved molecular elongation one residue at a time starting from the amino terminus for the first peptide and from the carboxyl terminus of the other. The conformers selected for further consideration at each step were those satisfying the arbitrary energy criteria of having an energy within 10 kcal/mol of the lowest minimum found, i.e. $\Delta U = U - U_{min} < 10$ kcal/mol. The starting points for energy minimization were the following backbone conformation sets: $\phi = -40°$, $\psi = -50°$; $\phi = 40°$, $\psi = 50°$; $\phi = 180°$, $\psi = 180°$ for MeA residues, $\psi = 140°$; $\psi = 80°$; $\psi = -60°$ for Pro residues, $\phi = -140°$, $\psi = 140°$; $\phi = -75°$, $\psi = 140°$; $\phi = -75°$, $\psi = 80°$; $\phi = -60°$, $\psi = -60°$; $\phi = 60°$, $\psi = 60°$ for other residues except Gly. For Gly, the latter set of five pairs of torsional values was extended by introducing the same pairs of angle values with opposite signs.

Having calculated the sets of low-energy conformers of both decapeptides, we used them for preparation of a set of starting conformations for the central decapeptide, 6–15. This set was formed by combination of all types of backbone conformations of pentapeptides 6–10 and 11–15 found in low-energy conformers of both amino- and carboxyl-terminal decapeptides. The set of stable conformers ($\Delta U < 10$ kcal/mol) of the 6–15 peptide was used in the same way, in combination with the data on the stable conformations of the amino-terminal decapeptide, for generation of starting conformers and elucidation of low-energy structures of the fragment 1–15.

As the final part of the vacuum calculations, the build-up procedure was applied again, with elimination of those starting structures in which the conformation of carboxyl-terminal part, beginning with residue 11, was not represented among the low-energy structures of decapeptide 11–20. 371 conformers of alamethicin were found which met the requirement that $\Delta U < 10$ kcal/mol.

Calculations at the phase boundary

A model

The phase boundary between the water and lipophilic phase is considered as a plane dividing two compartments with the solvation properties of water and octanol. A *constant* electric field is applied normally to the plane separating the phases, i.e., the potential profile has the linear form,

$$\Psi = \alpha Z \tag{1}$$

where Z is the coordinate normal to the phase boundary and the coefficient α (mV/Å) determines the strength of the electric field (Fig. 2). The transfer of a unity charge (expressed in e units) by 1 Å along Z results in the change of energy for the system of

$$10^{-3}|ev\,\alpha| = |0.0235\alpha| \text{ kcal/mol.} \tag{2}$$

In this simplified model, the phase boundary is considered as a surface at which water contacts a hydrophobic phase immediately, unlike real bilayers, or cell membranes, which have a charged polar layer (polar headgroups) between the two phases. Thus, the transmembrane-potential profile should differ from those discussed for artificial, or natural membranes (see McLaughlin (1989) and Cafiso (1991)). The choice of a linear form of the potential profile inside the lipophilic phase is stipulated by the Goldman constant-field theory which excellently describes most experimental data on the electrochemical properties of animal, plant, and bacterial cells at rest (see, e.g., Zachar (1971) and Yurin et al. (1977)). We assume that the electric field is linear also in the adjacent aqueous phase. This assumption simply reflect the lack of positive knowledge; but may be justified in part in terms of theory assuming the existence at the phase boundary of a water layer several angstroms thick, which is impenetrable for ions from solution and has a low dielectric constant, comparable to that of lipophilic phase (Krishtalik et al., 1991). As most stable conformers of the alamethicin molecule were calculated to be almost fully immersed into lipopholic phase with only a few functional groups on the aqueous side, this model appears reasonable. On the other hand, the side-chain carboxyl of the Glu^{18} residue, which was considered to be ionized, is usually protruding into water; its effective charge interacting with the applied electric field should be reduced due to screening

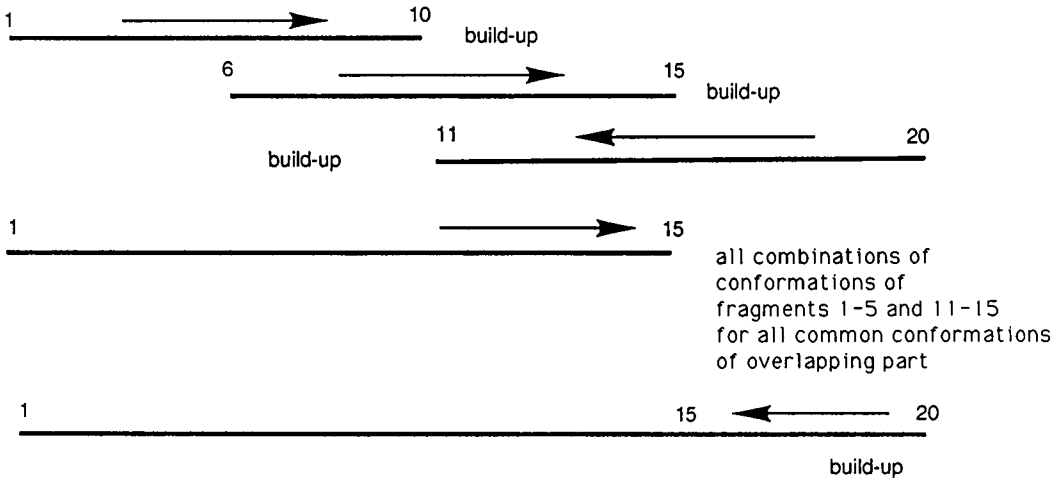

FIGURE 1 General scheme of calculation of stable alamethicin conformer in vacuo.

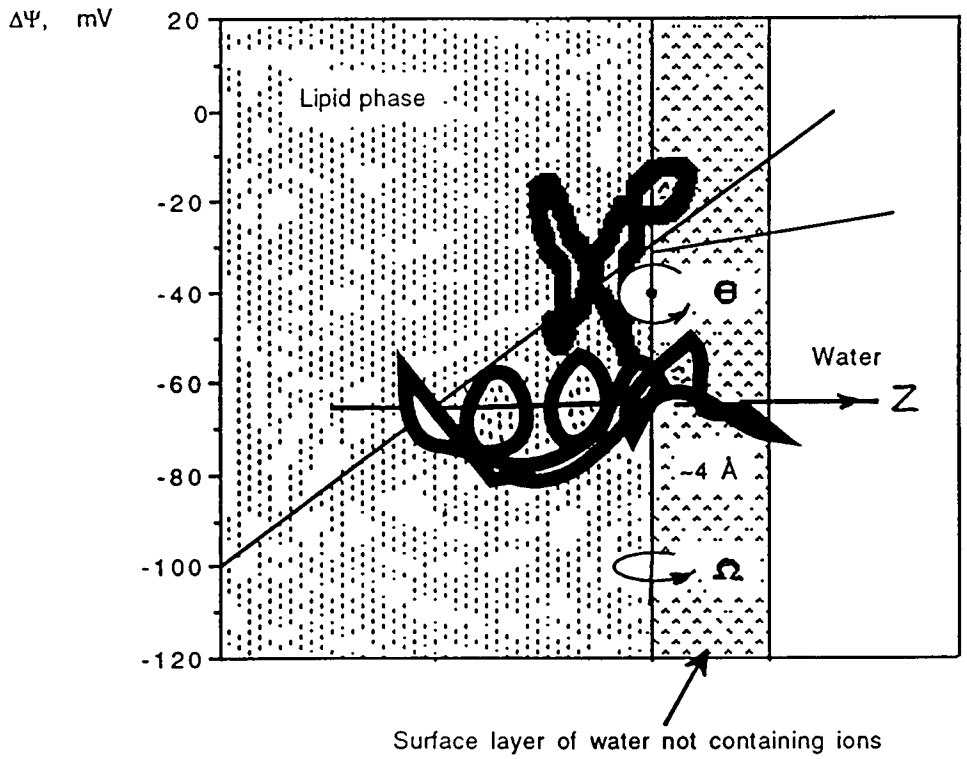

FIGURE 2 Symbolic molecule at the phase boundary. The electric field is applied across the boundary.

by counterions and other dielectric boundary effects which are difficult forquantitative evaluation (Matthew, 1985). To approximate these effects, we repeat the final stages of the calculations with the charges for the Glu side-chain proportionally reduced by the factor, 0.25. As all calculated structures have most of the molecule immersed into the lipophilic phase, the relative weights of ion-field and dipole-field interactions are regulated by varying the Glu effective charge. In this work, the effects of a 200-mV transmembrane potential have been investigated. Assuming that the lipid portion of the bilayer is approximately 30-Å thick (Wiener and White, 1992), the coefficient α assumes a value of 6.6 mV/Å.

Algorithms

The molecule was placed at the phase boundary, and the solvation energies of its components immersed in "water" and "lipophilic" phases were calculated using the corresponding parameterization. The Hopfinger-Scheraga continuum approach and parameters sets were used (Hodes et al., 1979; Hopfinger and Battershell, 1976). Despite the existence of recent refinements in methods for treatment of solvation effects in molecular mechanics (Ooi et al., 1987; Sharp et al., 1991; Still et al., 1990; Wesson and Eisenberg, 1992), we preferred this approach because of the availability of full sets of solvation parameters for both water and a lipophilic fluid (octanol) as a model of the membrane interior which has been extensively justified elsewhere; besides which, the parameterization of this model was compatible with the ECEPP/2 force field.

The effects of the electric field were considered by adding a third term, namely, the energy of interaction of the charged atoms with the electric field

$$U_{ef} = 0.0235\alpha \sum_i q_i Z_i \qquad (3)$$

where q_i, Z_i are the partial charge of ith atom and its distance to the phase boundary, respectively, to the sum of intramolecular and solvation energies. The latter value is positive on the "water" side and negative on the "lipid" side. The sum of the energy terms was minimized with respect to the internal torsional angles of the molecule, its translation in the direction normal to the phase boundary, and macrorotations with angles Ω and Q determining its orientation with respect to the phase boundary (Fig. 2). The energy minimization in water was carried out again with Fletcher-Powell-Davidon algorithm; at the phase boundary, where variables of different types are involved, the simplex algorithm (Nelder and Mead, 1965) proved to be more effective. The XY plane was considered to be the phase boundary; the subspace of macrorotation angles, Ω and Q, was scanned with a step size of 10°, using each combination of angles as a starting point for minimization.

All 371 conformers which were found most stable in vacuo were used as starting conformations for energy minimization, both in aqueous environment and at the phase boundary; no interim eliminations were done on the basis of the results of calculations in water. Out of the 371 starting structures, 49 interphase-bound structures met the arbitrary energy requirement that $\Delta U < 12$ kcal/mol.

All calculations were carried out using a modified version of CONSOMOL—a molecular mechanics program for the calculation of stable conformations at the phase boundary, described in more detail elswhere (Galaktionov et al., 1988).

Remarks on limitations of applied approach

Because of the size of the alamethicin molecule, one cannot systematically sample conformational space available to the molecule on a sufficiently fine grid to determine the conformational energy surface. The paradigm chosen, the build-up procedure, is a compromise between grid, or systematic sampling, and other procedures, such as molecular dynamics, which have limited sampling capability compared with the computational cycles expended. One obvious limitation of this study is the generation of a set of comformers (371) in vacuo which serve as starting points for minimization either in the aqueous model, or at the phase boundary. A second limitation concerns the discontinuous nature of the phase boundary model and its impact on the minimization results. Both of these limitations are admitted by the investigators, who view this study as both preliminary and exploratory to determine if such an approach might offer new insight into the behavior of peptides such as alamethicin at the lipid-water interface and calibrate possible effects of an imposed electric field. The results clearly indicate that such effects are significant and warrant exploration of these phenomena with more sophisticated models with greater atomic detail.

RESULTS AND DISCUSSION

Interphase-bound conformations in the absence of an electric field

Due to the pronounced hydrophobicity of the alamethicin molecule, the energies of transfer of each conformer from aqueous environment to the phase boundary are very large, ranging from −50.9 to −71.5 kcal/mol. Taking into account the difference in the hierarchy of stabilities of the final set of conformers in water and at the phase boundary, the evaluation of the energy of transfer of alamethicin molecule may be given in the form

$$\Delta U_{tr}^{min} = U_{b}^{min} - U_{w}^{min} \quad (4)$$

where U_{b}^{min} and U_{w}^{min} are energies of the conformers most stable at the phase boundary and in the aqueous environment, respectively. For the set of 49 conformers most stable at the phase boundary, the energy of transfer from aqueous environment to the phase boundary, $\Delta U_{tr} = -50.2$ kcal/mol.

For individual conformers, the energies of transfer clearly correlate with the amount of helical segments in a given conformer, with fully helical conformers having the largest transfer energy. The term "helical" is used here conditionally; in fact, the calculations did not elucidate any helical structures having values close to the canonical parameters of the α- or 3_{10}-helix despite the presence of many MeA residues which favor the formation of the latter type of helices; four helix-like structures were found, having all ϕ,ψ values negative (right-handed "helices"). All these conformers have very high energies in water (greater than 14 kcal/mol above the minima found in water), but are quite stable at the phase boundary (3.7–6.8 kcal/mol above the minima found). Direct experimental detection of this effect may be difficult due to the poor solubility of alamethicin in water; however, the increase of helix content after binding to lipid bilayers was observed for other peptides, e.g., glucagon (Kimura et al., 1992) and adrenocorticotropic hormone (Schwyzer, 1991). The conformations of bombolitin I and III have been studied by NMR and circular dichroism both in solution (Bairaktari et al., 1990a) and in association with micelles (Bairaktari et al., 1990b). Bombolitin I lacks any discernible structure in aqueous solution, while bombolitin III adopts an amphiphilic α-helix only at high concentrations consistent with molecular aggregation. When bombolitin I is bound to sodium dodecyl sulfate micelles, the α-helix induced extends from residues 3 to 15.

The importance of environment on the conformation of peptides has been underscored by recent results; on the cyclic 11-residue immunosuppressant peptide, cyclosporin A (see Marshall (1992) for review). The conformation in the crystal and in various organic solvents was known, and the conformation of cyclosporin complexed with its putative receptor, cyclophilin, has been determined. In nonpolar solvents or in the crystal, this hydrophobic peptide maximizes its internal hydrogen-bonding capability by forming a twisted β-sheet with one of its seven N-methyl amide bonds in the *cis* conformation. As the solvent becomes more polar (methanol or dimethyl sulfoxide), several other conformers due to amide-bond isomerism become populated. When bound to cyclophilin, all of the amide bonds have assumed the *trans* conformation, and the internal hydrogen bonds are broken in favor of a polar surface of amide hydrogens and carbonyls, some of which bind to cyclophilin. In effect, the structure has turned itself inside out in response to the polar environment. These results are consistent with the significant changes in relative stability seen for alamethicin conformers between the aqueous and phase boundary environments.

As the helical conformation is presumably important for alamethicin-induced, gating mechanisms, we characterized different conformers based on their proximity to helical structures; the number, N_R, of amino acid residues having negative backbone conformations, $\phi, \psi < 0$, was used as the measure of this proximity. In Table 1 are described the most stable representatives of the groups of conformers having 6, 9, 12, 17, and 19 residues in such conformations. Structures with 17 and 19 residues in helix-like conformations were the only samples of these classes in the above set of stable conformers; among the conformers with 6, 9, or 12 residues having both $\phi, \psi < 0$, the most stable representatives were chosen. Most conformers with N_R less then 10 to 12 had no helical segments with the residues in question dispersed along the structure (Fig. 3). It is noteworthy that some structures which were quite compact in vacuum became more loose in their lipid-immersed parts after the transfer to the phase boundary (Figs. 4 *a* and 5 *a*). Of four helical structures, conformer 5 most resembles the crystal conformation of alamethicin (Fox and Richards, 1982), though values of several backbone torsional angles[1] differ more then for 30°. The fragments Val-MeA, MeA-Pro-Val, and Leu-MeA in crystals of some model peptides (Karle et al., 1990a, 1990b) resembles very much in their backbone shapes the corresponding fragments of conformer 5, though differing in actual torsional angles. Conformer 4, in its amino-terminal part 1–9, is close to the conformation A1a of Furois-Corbin and Pullman (1988).

Effects of electric field

The changes in molecular orientation at the phase boundary induced by an electric field corresponding to transmembrane potential 200 mV are shown in part *b* of Figs. 4, 5, 6, 7, and 8. The application of this electric field changed the relative stabilities of two helix-rich conformers, 4 and 5, causing only minor shifts in the relative stabilities of the other conformers. Conformer 5 became the second most stable structure (0.4 kcal/mol). The fully helical conformer 5 in the absence of electric field is oriented so that the axis of the helix is almost normal to the phase boundary with the molecular dipole oriented along the helical axis, and its orientation is little influenced by the application of the electric field. The same is

[1] For crystal structure, torsional angles were calculated on the basis of atomic coordinates taken from the Brookhaven Protein Data Bank.

TABLE 1 The most stable representatives of helical, quasihelical, and nonhelical low-energy conformers of alamethicin: The interphase-bound conformation

Residue	Torsion angle, deg.	Conformer # 1	2	3	4	5
MeA[1]	ϕ	−52	−52	−51	−59	−57
	ψ	−53	−52	−53	−52	−52
Pro[2]	ψ	−40	−39	−43	−30	−35
MeA[3]	ϕ	53	53	54	−61	−64
	ψ	42	42	46	−28	−18
Ala[4]	ϕ	−160	−159	55	−81	−86
	ψ	−60	−60	51	−34	−36
MeA[5]	ϕ	53	53	−50	−60	−61
	ψ	44	44	−56	−29	−29
Ala[6]	ϕ	−78	−78	−130	−80	−77
	ψ	−44	−45	−66	−37	−33
	ϕ	−80	−80	−85	−66	−67
	ψ	147	143	154	−14	−24
Gln[7]	χ^1	−71	−69	−66	−84	−79
	χ^2	−179	179	168	176	−77
	χ^3	−19	−16	36	54	91
MeA[8]	ϕ	−49	−51	−46	−59	−65
	ψ	−43	−45	−45	−61	−36
	ϕ	−138	−140	−136	−67	−78
Val[9]	ψ	152	151	157	−27	−29
	χ^1	−76	−75	−74	−64	−176
MeA[10]	ϕ	−49	−48	51	−86	−59
	ψ	−46	−44	50	48	−40
Gly[11]	ϕ	−97	−94	−60	−136	−80
	ψ	73	77	−44	−65	−26
	ϕ	−94	−87	−97	−96	−92
Leu[12]	ψ	86	92	143	27	−14
	χ^1	−167	−161	−74	−57	−49
	χ^2	79	74	159	116	113
MeA[13]	ϕ	−52	−52	52	−80	−61
	ψ	−53	−52	54	−54	−53
Pro[14]	ψ	91	100	−37	−60	−42
	ϕ	−60	−137	−72	−49	−58
Val[15]	ψ	147	152	−29	−29	−27
	χ^1	−67	−79	−64	−48	178
MeA[16]	ϕ	52	57	−63	−63	−64
	ψ	46	45	−43	−51	−46
MeA[17]	ϕ	−62	53	−58	−55	−65
	ψ	−32	46	−42	−28	−18
	ϕ	−66	49	−52	−64	−72
	ψ	−41	73	−46	−49	−42
Glu[18]	χ^1	−75	−66	−73	−73	−75
	χ^2	176	178	173	−76	−74
	χ^3	0	4	178	165	152
	ϕ	−54	−138	−76	−53	−60
	ψ	−44	86	147	−42	−46
Gln[19]	χ^1	−76	−78	−64	−79	−67
	χ^2	178	168	164	−74	−68
	χ^3	28	29	46	100	104
	ϕ	−147	−153	−153	−100	−127
	χ^1	44	41	46	−52	−68
Phol[20]	χ^2	80	76	77	105	92
	θ^1	83	60	60	58	65
	θ^2	90	91	90	89	89
Energy in different environments, kcal/mol	Vacuum	4.5	5.4	0.0	10.6	8.9
	Water	5.0	5.3	9.8	17.2	17.4
	Phase boundary, 0 mV	2.1	2.7	0.0	5.9	3.7
	Phase boundary, 200 mV	2.3	2.9	0.0	4.6	0.4

true in relation to conformer 4 with both its large helical segments oriented more or less along the electric field gradient. On the contrary, the orientation of the conformers with little or no helical content changed dramatically. This reorientation was accompanied also by some changes in conformation. As it is mentioned above, the energy of their interaction with lipid phase is much less then that of helical conformers; therefore, they are easier moved into the aqueous phase by the force arising from the interaction of the charged Glu[18] carboxyl group with the field. The moderation of the effective charge on this group lessened the field-induced effects; however, they are still appreciable. Reduc-

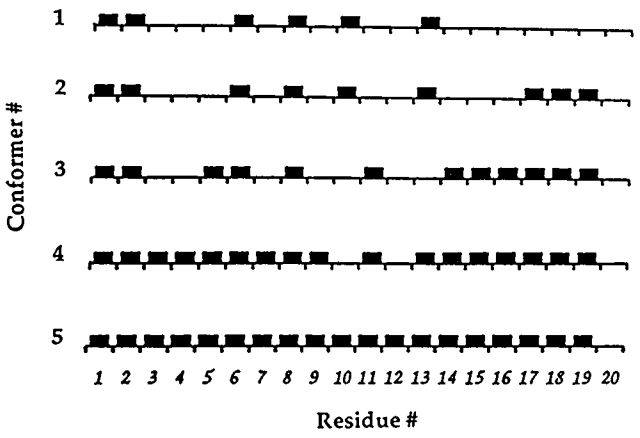

FIGURE 3 Positions of the residues with backbone conformation $\phi,\psi <$ 0 in five conformers described in Table 1.

tion of the charge 4-fold did not cause any significant changes in the hierarchy of stabilities. The NMR data of Esposito and co-workers (Esposito et al., 1987) did not show any influence of ionization of the carboxyl group on the overall conformation of alamethicin in methanol, although the environments are sufficiently different to preclude direct comparison of the two effects. Fig. 9 illustrates the relative stabilities of the five conformers described in Table 1 for different environment models.

Some implications for understanding binding and gating mechanisms

Fig. 10 presents schematically the network of events presumably involved in the mechanism of voltage-dependent gating by alamethicin on lipid bilayers. All hypothetical mechanisms proposed for the explanation of alamethicin's

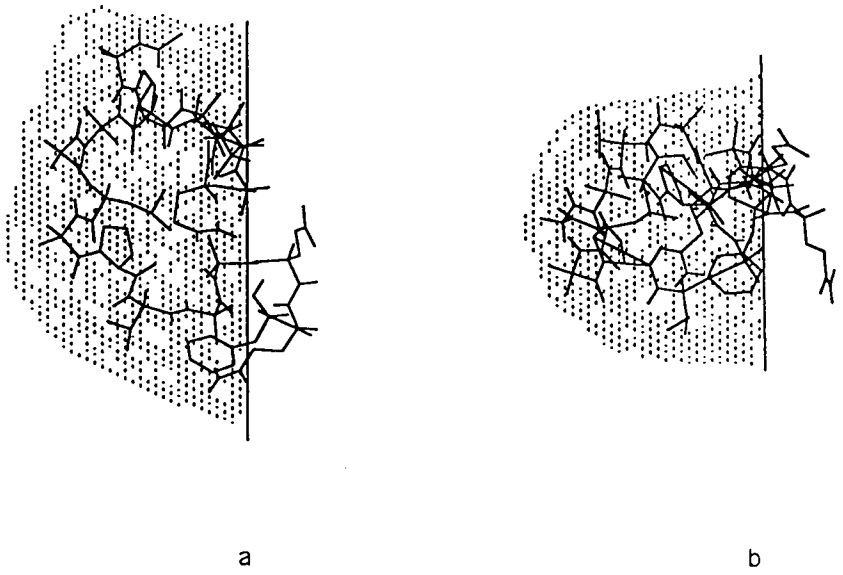

FIGURE 4 Conformer 1 at the phase boundary. *a*, without electric field; *b*, electric field 200 mV.

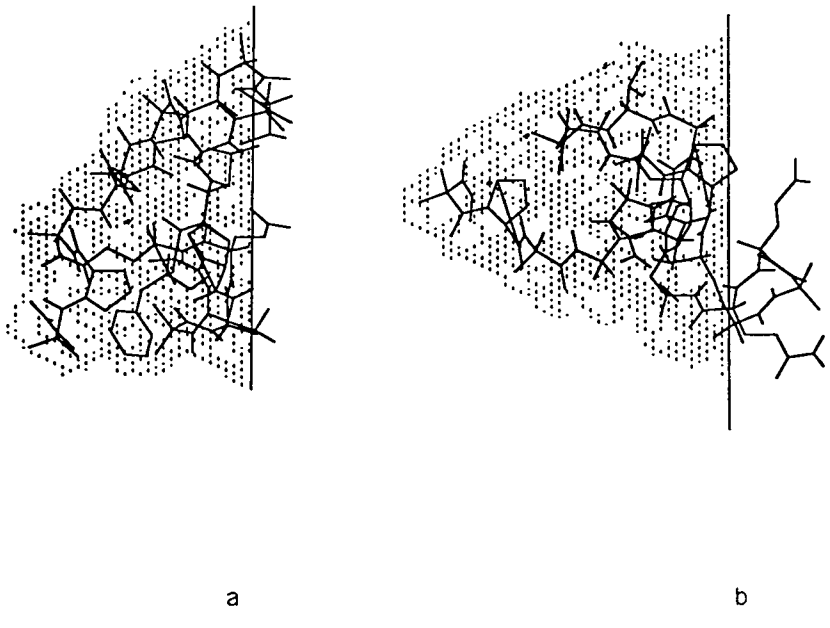

FIGURE 5 Conformer 2 at the phase boundary. *a*, without electric field; *b*, electric field 200 mV.

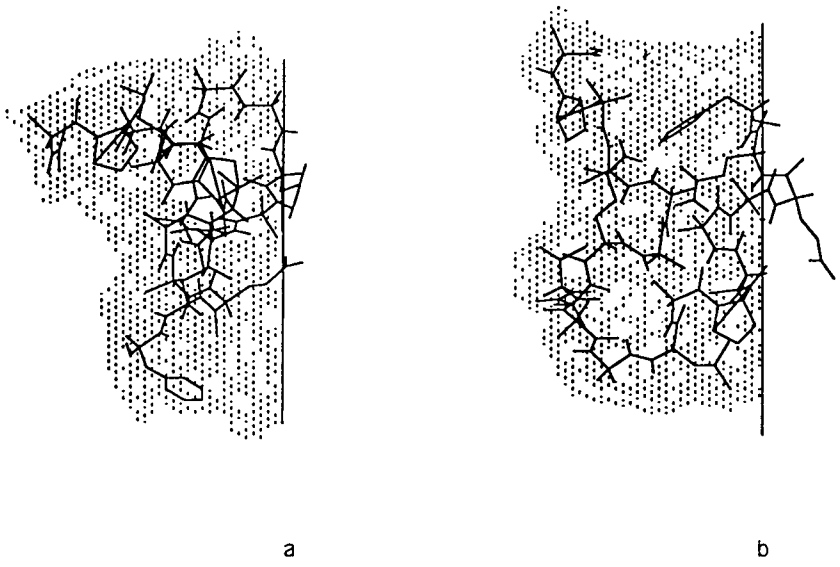

FIGURE 6 Conformer 3 at the phase boundary. *a*, without electric field; *b*, electric field 200 mV.

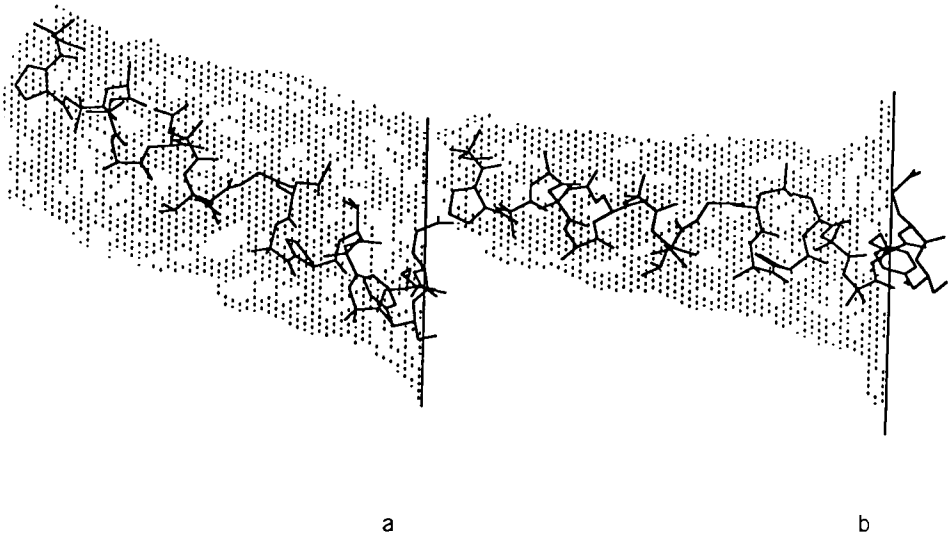

FIGURE 7 Conformer 4 at the phase boundary. *a*, without electric field; *b*, electric field 200 mV.

gating activity (see Marshall and Beusen (1992) and Sansom (1991) for review) are in fact different pathways through this network determining some set of options based on a given experimental paradigm. Let us use the computational results to comment on several elements of this network. It seems unlikely that any structure stable in the aqueous environment at low concentrations due to solubility might be significantly represented in the ensemble of interphase-bound conformers.

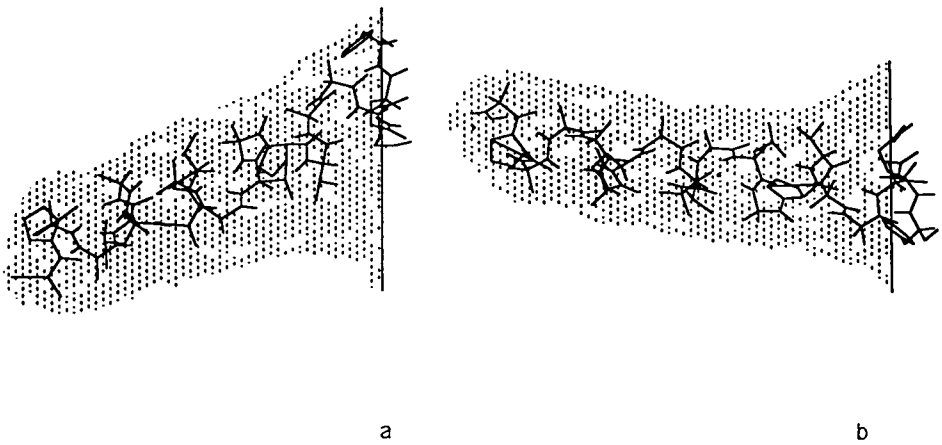

FIGURE 8 Conformer 5 at the phase boundary. *a*, without electric field; *b*, electric field 200 mV.

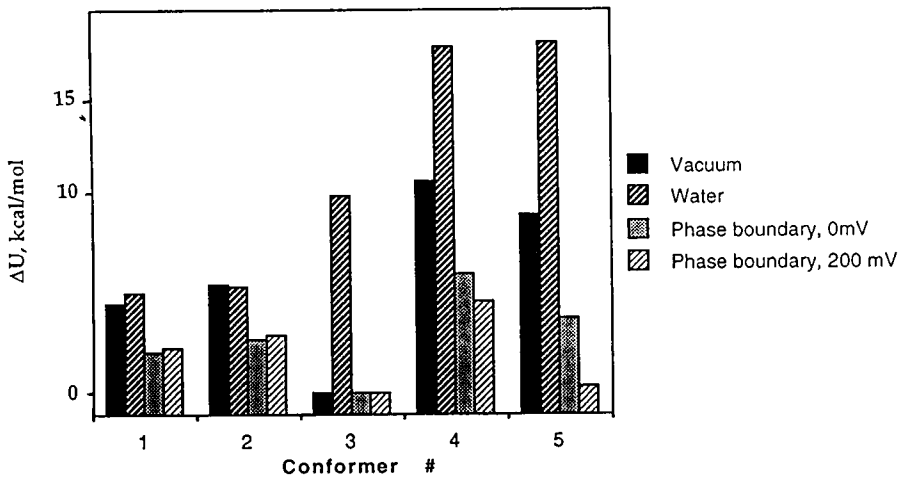

FIGURE 9 The relative stabilities of the five conformers described in Table 1 in four environments.

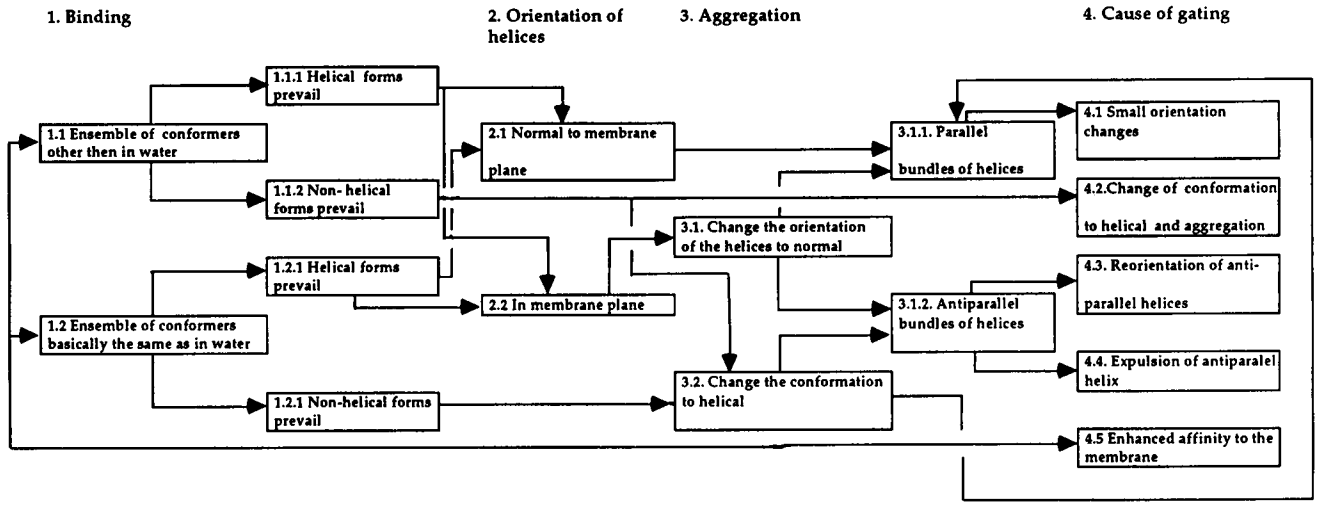

FIGURE 10 The network of events presumably involved in the gating effect.

The conformers which are stable in the aqueous environment have high energies at the phase boundary, e.g., 11.8 kcal/mol for the lowest-energy aqueous structure (Fig. 11). Conversely, the interphase low-energy conformers, especially those with high helix content, are totally unstable in water. Therefore, the path associated with option 1.2 of the network (Fig. 9) can be eliminated.

The application of the electric field makes the energies of transfer from aqueous environment to the phase boundary slightly more negative in the case of the helical structures (3.3 kcal/mol for conformer 5). Thus, option 4.5 cannot be eliminated on the basis of these results; however, experimental data on the gating effect in vesicle suspensions, where practically all of the peptide is in the bound state (Archer and Cafiso, 1991), seem to contradict the partitioning hypothesis (Schwarz et al., 1986).

A choice between options 1.1.1 and 1.1.2 is impossible based on the computational results; both helical and nonhelical structures are represented in the set of interphase-bound, low-energy conformers. Although the most stable structure is nonhelical, the best of the fully helical-like conformers has an energy only 3.7 kcal/mol less stable. Spectroscopic studies have suggested the existence of nearly equipopulated helical and nonhelical fractions of membrane-bound alamethicin; the relative percentage depends strongly on many physical and chemical factors, first of all membrane hydration (Vogel, 1987).

Furthermore, helical and quasihelical conformers have their helices immersed into the lipophilic phase in such a way that the angles between the helix axes and the phase-boundary plane are all above 60–70°; the application of the electric field increases these angles still closer to 90°. Much experimental data exists suggesting deep insertion of the alamethicin amino terminus into the lipid core of a membrane rather then binding of the molecule parallel to the lipid-water interface suggested by an earlier study (Banerjee et al., 1985); again, the orientation depends strongly on the physical state of the bilayer (Sansom, 1991; Vogel, 1987; Huang

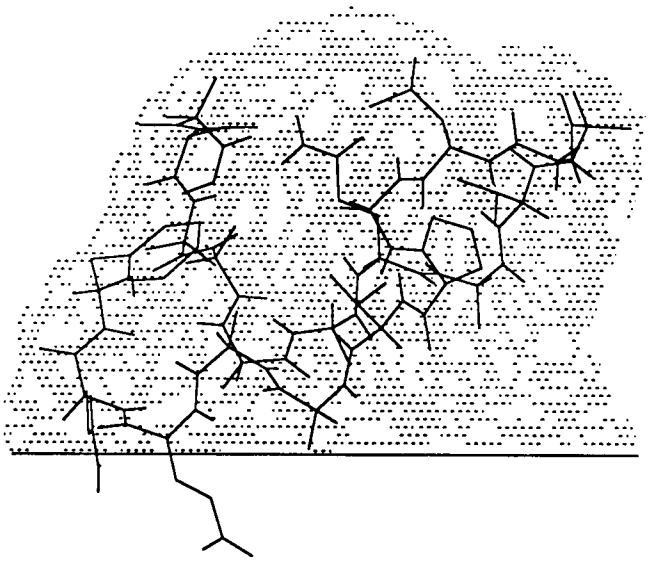

FIGURE 11 The conformer having lowest energy in water bound at phase boundary.

and Wu, 1991; Stankowski and Schwarz, 1989). Therefore, option 2.1 is preferable based on the combination of evidence of experimental data with the results of these calculations.

Although aggregation phenomena themselves were not the subject of this study, the results suggest some comments on the possibility of formation of bundles of antiparallelly oriented helices and their reorientation by the electric field. Namely, the transfer of the Glu^{18} carboxyl group across the lipid core of the membrane, which is necessary for such reorientation needs to overcome a potential barrier of more then 15 kcal/mol; the facilitating influence of 200-mV electric field can be estimated to contribute a maximum of 4.6 kcal/mol (energy of transfer of unity charge across a 30-Å thick membrane with transmembrane potential 200 mV). In fact, alamethicin has been shown to be unable to cross many lipid bilayers; moreover, its application to one side of such a bilayer results in strongly asymmetric current-voltage curves (Hall et al., 1984). Analogs which lack the carboxyl functionality of glutamic acid show symmetric current-voltage curves. Taking into account these observations, elimination of option 3.1.2 and, hence, the final options 4.3 and 4.4 from mechanistic consideration appears warranted.

The remaining options 4.1 and 4.2 both seem to be feasible; the application of the electric field induces a significant increase in the population of helical and quasihelical conformers as well as the reorientation of the helices so that they become a bit more perpendicular to the interphase plane. The energetic effects of such reorientation are negligible (some 0.2–0.4 kcal/mol), however, and are easily obtainable by macrorotations, being almost independent from other variables. The observation of Wille and co-workers (Wille et al., 1989) which found that transmembrane potential forced the carboxyl terminus of alamethicin deeper into membrane core may be interpreted as evidence of such reorientation and support of option 4.1. In support of option 4.2, a potential-induced increase of helicity of membrane-bound alamethicin was suggested by Brumfield and Miller (1990) on the basis of the data of circular dichroism measurements.

CONCLUSIONS

An initial effort to determine the environmental effect on the conformational ensemble of alamethicin has shown significant changes in the relative energetics of alamethicin conformers as they move from aqueous solution to the lipid-water interface. Furthermore, inclusion of a crude model of the electrostatic potential has indicated a capacity to influence the distribution of conformers as well as their orientation. Despite the view prevalent in the literature, nonhelical conformers of alamethicin appear to play a significant role in the conformational ensemble and cannot be ignored in either energetic or possible mechanistic considerations. Analysis of the many different mechanistic pathways proposed for voltage-dependent gating by alamethicin reveal several which are inconsistent with the general trends discovered by these preliminary studies. Such studies may be useful to generate appropriate starting configuration for more detailed simulations of lipid bilayers (Brasseur, 1986; Charifson et al., 1990; De Loof, 1991; Damodaran et al., 1992; Egberts, 1988; Kroll and Gompper, 1992) where atomic details are included.

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