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Rapid report

Ion channel stability and hydrogen bonding Molecular modelling of channels formed by synthetic alamethicin analogues

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Abstract

Several analogues of the channel-forming peptaibol alamethicin have been demonstrated to exhibit faster switching between channel substates than does unmodified alamethicin. Molecular modelling studies are used to explore the possible molecular basis of these differences. Models of channels formed by alamethicin analogues were generated by restrained molecular dynamics in vacuo and refined by short molecular dynamics simulations with water molecules within and at either mouth of the channel. A decrease in backbone solvation was found to correlate with a decrease in open channel stability between alamethicin and an analogue in which all α-amino-isobutyric acid residues of alamethicin were replaced by leucine. A decrease in the extent of hydrogen-bonding at residue 7 correlates with lower open channel stabilities of analogues in which the glutamine at position 7 was replaced by smaller polar sidechains. These two observations indicate the importance of alamethicin/water H-bonds in stabilizing the open channel. © 1997 Elsevier Science B.V.

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Ion channels are found in the cell membranes of all organisms, regulating their electrical and other activities. A molecular understanding of their structure-function relationships is hindered by the paucity of information concerning their structures. In contrast, the structure-function relationships of channel-forming peptides (CFPs) [1] may be more readily dissected as structures for a number of CFPs have been determined at atomic resolution.

Alamethicin (Alm), a 20 residue peptaibol from the fungus *Trichoderma viride* [2], is perhaps the best characterization of the CFPs. Alm contains the strongly helix-promoting [3] residue α-amino-isobutyric acid (Aib), and a phenylalaninol at its C-terminus (Table 1). The crystal structure of Alm has been determined to atomic resolution [4]. The monomer is mostly α-helical, containing a kink induced by a proline at residue 14, an amphipathic α-helical N-terminal section and a more polar but less regular C-terminal helix [4]. Alm provides a good model system for the investigation of ion channel properties and of peptide-bilayer interactions.

The channel properties of Alm are well characterised [5–9]. Transient channels are formed in bursts,

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in a voltage-dependent manner [10]. During an open channel burst, the single-channel conductance switches between a number of distinct levels. Conductance levels increase and decrease in unit steps and each burst, usually both, starts and ends from the first conductance level. Conductance levels are not integral multiples of a fundamental conductance, rather the difference between adjacent levels increases as one progresses to higher conductances.

The 'barrel-stave' [11] or 'helix-bundle' [12] model provides the most persuasive explanation of the multiple conductance levels of Alm. In this model, a number (N) of Alm helices are packed together, in an approximately parallel fashion, around a central ion permeable pore. Each distinct conductance level is believed to correspond to a different number of Alm monomers within the channel aggregate. Thus, an increase or decrease in conductance level corresponds to addition or removal of an Alm monomer from the channel. There is considerable evidence supporting the assumptions implicit in this model [7,8]. The assumption that the constituent monomers of a pore-forming bundle adopt an α-helical conformation similar to that observed in the X-ray [4] and solution NMR [13] structures is justified by much spectroscopic data which suggest that Alm retains its α-helical conformation when interacting with lipid bilayers [14–16]. The assumption that Alm molecules form a transmembrane helix bundle around a central aqueous pore is supported by in-plane neutron scattering data [17]. That the constituent helices of the bundle are parallel (rather than anti-parallel) helices is suggested by: (i) the pronounced asymmetry of Alm current–voltage curves (reviewed by e.g. [8]); (ii) electrophysiological studies of Alm channel block by polycations [18]; and (iii) the demonstration that channels formed by covalently-linked parallel dimers of Alm helices resemble those of the parent Alm channels in their conductance values [19]. A further aspect of the helix-bundle model is that the hydrophilic surface of the Alm helices (defined by residue Gln-7) form the lining of the pore. This is reasonable on energetic grounds. The hydrophilic face of a helix will prefer to face the aqueous lumen of a pore rather than the surrounding hydrophobic fatty acyl chains [20].

One implication of the helix-bundle model is that the stability of a bundle for a given value of N may be expected to determine the mean lifetime of the corresponding single channel conductance level. Thus, the relative stabilities of helix bundles (i.e. the strength of the interactions between the monomers within an Alm bundle and between the bundle and its environment) are predicted to determine the relative open channel stabilities of different Alm analogues.

This prediction has been investigated with reference to a series of designed Alm analogues in which all Aib residues were replaced by leucine [21–24]. Particular attention has been focused on residue 7, which has been systematically altered [25] to produce a series of analogues (Table 1). This residue (Q7 in Alm) is the sole polar residue in the N-terminal half of the Alm monomer and has been suggested to play a key role both in ion permeation and channel stabilization [4,25]. Functional characterization of these analogues revealed a progressive decrease in open

Table 1
Synthetic analogues of alamethicin

Peptide	Relative open channel stability	Sequence
		3 7 10 14
Alm	8.7 (±2.5)	Ac-U-P-*U*-A-U-A-***Q***-U-V-*U*-G-L-U-*P*-V-U-U-E-Q-Phl
Alm-dUL	1.0	Ac-L-P-*L*-A-L-A-***Q***-L-V-*L*-G-L-L-*P*-V-L-L-E-Q-Phl
Alm-Q7N	0.92 (±0.16)	Ac-L-P-*L*-A-L-A-***N***-L-V-*L*-G-L-L-*P*-V-L-L-E-Q-Phl
Alm-Q7S	0.67 (±0.07)	Ac-L-P-*L*-A-L-A-***S***-L-V-*L*-G-L-L-*P*-V-L-L-E-Q-Phl

The Alm sequence is that of the R_f30 form. Residue 7 is shown in a bold italic font. Other residues of interest (3, 10, 14) are shown in italic.

Ac – acetyl moiety; U – Aib; Phl – phenylalaninol. Relative open channel stabilities are expressed as the average over the first four conductance levels of the mean open channel lifetime of the peptide relative to that of Alm-dUL. For experimental details see [25].

channel stability (i.e. increase in the rate of switching between adjacent conductance levels) from Alm-dUL to Alm-Q7S [25], i.e. as the polar sidechain was shortened, as summarised in Table 1. In this study we attempt to explain the pattern of open channel stabilities by molecular modelling of possible structures for the channels formed by these peptides.

Molecular models were generated using simulated annealing via restrained molecular dynamics (SA/MD) run using Xplor [26] version 3.1. The models of channels formed by the derivatives of Alm were generated in the same manner and embodied the same underlying assumptions (see above) as for models of native Alm channels described in [27]. SA/MD and subsequent solvation and refinement of the models by a short molecular dynamics (MD) simulation was performed as in previous studies unmodified Alm channels [27,28]. Briefly, ensembles of 25 models were generated using in vacuo SA/MD [29]. A representative member from each ensemble was solvated using Quanta/Charmm. The system was then energy minimised, heated to 300 K, equilibrated for 9 ps and a short dynamics run of 60 ps performed, with coordinate sets saved every 0.5 ps. During the MD simulations, restraints were applied to the protein atoms of the system to mimic the effects of the bilayer and to the waters to prevent their 'evaporation' from the pore mouths. MD simulations used Charmm [30] version 23f3, with the Charmm param19 parameter set, with only polar hydrogen atoms explicitly represented. The water model used was a TIP3P three-site model [31] with partial charges $q_O = -0.834$ and $q_H = +0.417$. Simulations were run on a DEC 2100 4/275. Structures were visualised using Quanta V4.0 (Biosym/Molecular Simulations) and drawn using Molscript [32]. The 120 models created during each MD simulation were analyzed in terms of their helix-bundle geometry and their hydrogen bond patterns. A distance cut-off of 3.0 Å and a minimum energy of −0.05 kcal/mol were used to define hydrogen bonds.

Channel models were generated with from $N = 6$ to $N = 9$ helices per bundle. In this paper, we present a comparative analysis of the $N = 6$ bundles as representative of the properties of these models. Similar results are seen from analysis of the $N = 7$, 8 and 9 bundles (not shown). The conformations of Alm and Alm-dUL channel models (with $N = 6$ helices per bundle) at the end of the 60 ps MD in the presence of water are shown in Fig. 1. As the three synthetic Alm analogues (i.e. Alm-dUL, Alm-Q7N and Alm-Q7S, see Table 1) yielded helix bundles with similar conformations only that of Alm-dUL is illustrated. The arrangements of the helices in the two channel models are distinctly different. In the Alm model, the mean crossing angle is +18°, whereas in Alm-dUL it is −1.5°. Thus, whereas the Alm bundle exhibits a clear left-handed supercoil, in the Alm-dUL bundle there is little, if any, coiled-coil structure. Thus, although the replacement of Aib by Leu is to a first approximation a conservative substitution, it does appear to have subtle effects on the packing of the helices.

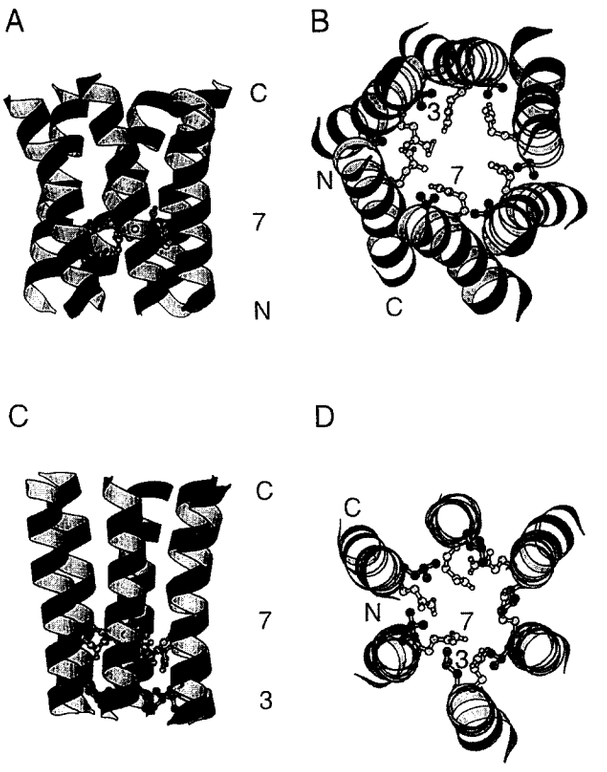

Fig. 1. Models of the Alm and Alm-dUL $N = 6$ channels. Models are drawn using Molscript [32] with the helix backbones depicted as ribbons. Residues 3 (dark grey) and 7 (light grey) are shown in ball-and-stick representation. For clarity, pore waters have been omitted. (A,B) Alm; (C,D) Alm-dUL. In (A,C) the channel models are viewed perpendicular to the (presumed) bilayer normal; in (B,D) the view is down the bilayer normal, with the N-termini of the helices towards the viewer.

Hydrogen-bonding interactions of the luminal water molecules with the channels differ significantly between Alm and its synthetic analogues and also differ between the various analogues. The first difference lies in the number of hydrogen bonds formed by residue 7 in three synthetic analogues, i.e. Alm-dUL, Alm-Q7N and Alm-Q7S. If one defines an 'X7 H-bond pair' as either a residue 7/residue 7′ H-bond (where the ′ indicates a neighbouring helix in a bundle) or a residue 7/water/residue 7′ H-bond network, then the number of X7 H-bond pairs formed during the 60 ps MD simulations decreases as the polar sidechain at position 7 is shortened (Fig. 2). In particular, there are consistently more X7 H-bond pairs in the Alm-dUL and Alm-Q7N models than in Alm-Q7S. Furthermore, all X7 H-bond pairs in the Alm-Q7S model simulation are 'indirect', i.e. mediated by a water molecule rather than via a direct Ser 7/Ser 7′ interaction. More 'direct' X7 pairs, and more X7 pairs in total, are observed for Alm-dUL than for Alm-Q7N, although the difference between these two systems is not as marked as that between these systems and Alm-Q7S. This trend in H-bonding patterns correlates quite clearly with the open channel stabilities of the three analogues. In particular, Alm-dUL and Alm-Q7N have significantly greater open channel stabilities than does Alm-Q7S. Thus, it appears that H-bonding via formation of X7 pairs stabilizes the open state of the channel.

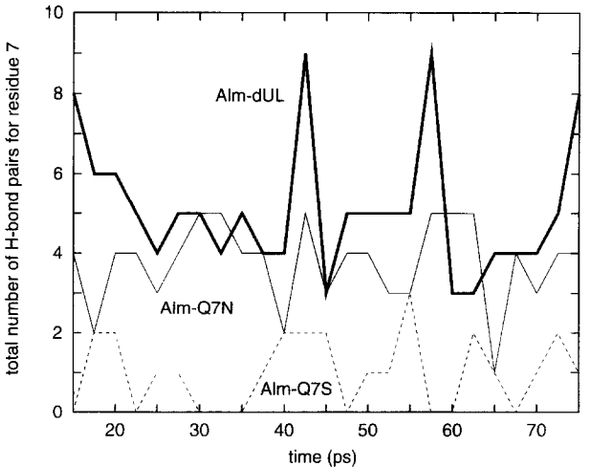

Fig. 2. Numbers of X7 hydrogen-bonded pairs. Numbers of total X7 pairs (direct and indirect) averaged over the 60 ps MD runs for the three synthetic analogues are given (Alm-dUL = bold line; Alm-Q7N = thin line; Alm-Q7S = broken line).

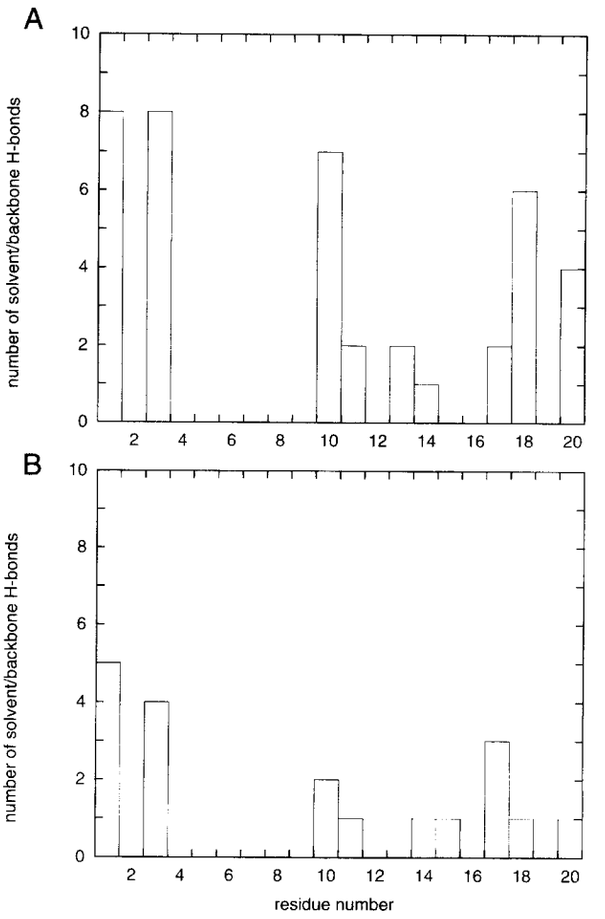

Fig. 3. Backbone solvation frequency, residue by residue. The mean numbers per bundle of water-to-backbone H-bonds ('frequency of backbone solvation'), averaged over the final 25 ps, are shown for simulations (A) Alm and (B) Alm-dUL.

A second difference in H-bonding emerges if one compares the pattern of solvation (i.e. H-bond formation to water) of the polypeptide backbone of the Alm bundle with those of channels formed by the synthetic analogues. The Alm-dUL, Alm-Q7N and Alm-Q7S bundles all displayed a lesser degree of backbone solvation of pore-lining residues from that observed in the Alm channel models, as summarised in Fig. 3. This difference in solvation is most pronounced for residues where a pore-lining Aib residue in Alm has been substituted by a leucine in Alm-dUL,

e.g. residues 3 and 10. The bulkier leucines appear to shield the helix backbone from the pore waters to a greater extent, thus reducing the extent of solvation. Of course, it must be remembered that restraints during the simulations maintained the integrity of the helices (in accordance with spectroscopic data), but despite such restraints it is evident that the Aib-to-Leu substitution produces a decrease in pore solvation.

Before the implications of this study are discussed, the reliability of the computational strategy should be evaluated. The molecular modelling methodology used has been employed to model a number of channel systems [33–36]. The underlying assumptions as to the nature of the Alm channel (e.g. the approximately parallel orientation of the α-helices) have been discussed in detail [27] and are justified in the light of the available experimental data [19,27,37,38]. The SA/MD technique used to generate our initial models has been used successfully in protein structure prediction [39], and has been applied to modelling channels in combination with experimentally-derived structural restraints [40,41]. The solvation and solvated modelling methodology has also been extensively discussed and seems to yield reasonable results for Alm and other CFP channels [28]. Similar methods have been applied by other investigators to a number of other channel systems [42–44]. Thus, whilst bearing in mind the limitations of any modelling study, it seems likely that our results provide a reasonable model of the underlying channel structures.

We observe two classes of correlation between the hydrogen-bonding properties of Alm channel models and the observed open channel stabilities of the synthetic analogues. Assuming that changes in conductance level occur as described by the helix-bundle model, then each switch in conductance level represents a change in the molecularity of the channel aggregate from N to $N \pm 1$. It is reasonable to propose that this may involve a transition state in which a helix is either halfway inserted or removed from the bundle. By this we imply a transition state in which a helix is about to leave/enter the bundle via lateral diffusion in the plane of the membrane. Thus, the leaving/entering helix in such a transition state will have broken/not formed any H-bonds it forms with neighbouring helices when within a bundle. This transition state is likely to be less stable than either the N or the $N \pm 1$ state. The difference in stability between the transition state and its neighbouring N and $N \pm 1$ states will determine the rate of switching between the two corresponding conductance levels. Thus, changes in the interactions within Alm channels that reduce this difference in stability between the open channel states and the intervening transition states may be expected to increase the rate of switching between adjacent conductance levels, and hence decrease the relative open channel stability.

The Alm-dUL and Alm-Q7N analogues exhibit greater open channel stabilities than the Alm-Q7S analogue (Table 1). Furthermore, the Alm-dUL and Alm-Q7N channel models exhibit a higher number of X7 H-bond pairs than the Alm-Q7S model. 'Rings' of hydrogen bonds formed by the Q7 residues of Alm have been proposed to be important for channel stability [4]. The insertion/removal of a helix from an Alm bundle would transiently disrupt such hydrogen bond networks. Such disruption will increase the energetic cost of insertion/removal and thus reduce the relative stability of the transition state. Therefore, the stronger the inter-helix hydrogen bond network of the channel states for an analogue, the greater the difference in stability between the transition state and the channel states and, hence, the greater the relative stability of the open channel. The Alm-Q7S analogue, which has a much lesser degree of inter-helix hydrogen bonding, would thus be expected to exhibit a significantly lower open channel stability than the Alm-dUL and Alm-Q7N analogues, which have stronger inter-helix hydrogen bonding. This is exactly what is observed experimentally, thus supporting this model of channel formation.

A similar argument holds for the relationship between the degree of peptide backbone solvation and the relative open channel stabilities of Alm and Alm-dUL. These two forms of Alm both have glutamine at residue 7 and exhibit similar numbers of Q7 H-bonded pairs in the MD simulations. However, the level of backbone solvation is much higher for the Alm bundle. Thus, the favourable interactions between the channel and its environment (i.e. luminal water molecules) are reduced for Alm-dUL relative to Alm. The stability of the Alm-dUL channel open states is thus predicted to be less than that for Alm. Again, this matches the observed single channel data, thus

supporting the proposed model. It is also possible that a small difference in the length of the helix between Alm-dUL and Alm may have an effect on channel lifetimes [45].

Of course, these arguments do not take into account possible differences in interactions of the Alm analogues with the lipid bilayer. Such differences, if they exist, are not thought likely to have a significant effect on relative channel lifetimes. Among the Q7X Alm analogues, the residues facing the bilayer are identical; the strength and nature of the interactions of these residues with the bilayer may thus reasonably be expected to be similar in all three cases. There are differences in the composition of the bilayer face of the channel for Alm and Alm-dUL. However, following the arguments put forward in [46], we have concentrated on specific interactions between transmembrane helices to explain the stability of their aggregates as there appear to be no significant non-specific forces which drive transmembrane helix association. The nature of the peptide/bilayer interactions of Alm channels will be addressed in further simulations. The degree of agreement between experiment and simulation revealed in the current studies encourages one to believe that such more detailed (and more computationally demanding) simulations will prove well founded, and will provide a complete description of the nature of this 'simple' ion channel.

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