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The Peptaibol Antiamoebin as a Model Ion Channel: Similarities to Bacterial Potassium Channels‡

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Abstract: Antiamoebin (AAM) is a polypeptide antibiotic that is capable of forming ion channels in phospholipid membranes; planar bilayer studies have suggested the channels are octamers. The crystal structure of a monomeric form of AAM has provided the basis for molecular modelling of an octameric helical bundle channel. The channel model is funnel-shaped due to a substantial bend in the middle of the polypeptide chain caused by the presence of several imino acids. Inter-monomer hydrogen bonds orientate a ring of glutamine side chains to form a constriction in the pore lumen. The channel lumen is lined both by side chains of Gln11 and by polypeptide backbone carbonyl groups. Electrostatic calculations on the model are compatible with a channel that transports cations across membranes.

The AAM channel model is compared with the crystal structures of two bacterial (KcsA and MthK) potassium channels. AAM and the potassium channels exhibit common functional features, such as cation-selectivity and similar single channel conductances. Common structural features include being multimers, each formed from a bundle of eight transmembrane helices, with lengths roughly comparable to the thickness of lipid bilayers. In addition, they all have aromatic amino acids that lie at the bilayer interfaces and which may aid in the stabilization of the transmembrane helices, as well as narrower constrictions that define the ion binding sites or selectivity filters in the pore lumen. The commonality of structural and functional features in these channels thus suggests that antiamoebin is a good, simple model for more complex bacterial and eukaryotic ion channels, capable of providing insight into details of the mechanisms of ion transport and multimeric channel stability.

Keywords: conductance; crystal structure; ion channel; potassium channel; molecular model; alamethicin

INTRODUCTION

Antiamoebin (AAM) is a 16-residue antibiotic polypeptide (Figure 1) isolated from the fungus *Emercelliopsis poonensis* [1]. It is capable of inserting into lipid bilayers and conducting ions across membranes [2], functions that result in its antibiotic properties.

Antiamoebin is a member of the peptaibol family of membrane-modifying peptides. These peptides are characterized by a high content of

Antiamoebin-I Ac-F-U-U-U-J-G-L-U-U-***O***-**Q**-J-***O***-U-***P***-F-OH

Figure 1 The sequence of AAM. Residues in italics are imino acids, residues in bold have hydrophilic side chains. Ac, acetyl; U, α-aminoisobutyric acid; J, isovaline; O, hydroxyproline (Hyp).

α,α-substituted amino acids, e.g. α-aminoisobutyric acid and isovaline. These types of residues restrict, due to steric hindrance, the range of φ, ψ angles their polypeptide backbones can adopt, predisposing them to helical secondary structures [3]. The extra α-carbon substituents also tend to increase their hydrophobicity, thereby contributing to their ability to insert into lipid bilayers. Most peptaibols also have 'blocked' termini: acetylated *N*-termini

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and *C*-terminal amino alcohols, so they cannot form zwitterions, thus further increasing their hydrophobicity. It has been proposed that the long peptaibols form transmembrane ion channels by association of monomers into helical bundles [4–6]. The sequences of over 300 peptaibols, including 16 homologues of antiamoebin, can be found in the peptaibol database [7,8] located at http://www.cryst.bbk.ac.uk/peptaibol.

Single channels of antiamoebin with single-level bursts of conductance and marginal voltage-dependence have been observed in planar lipid bilayers composed of palmitoyloleoyl phosphatidylcholine (POPC) and dioleoyl phosphatidylcholine (DOPC), albeit at very high peptide concentrations [2]. AAM does not appear to form channels in a number of other lipids, such as glycerol monooleate or diphytanoyl phosphatidyl choline [9].

The crystal structures of a number of peptaibols have been determined, including alamethicin [10], antiamoebin-I [9,11], Leu-zervamicin [12], and trichotoxin_A50E [13]. In all cases, crystals have been prepared from organic solvents. The molecular forms present in the crystals are monomers, rather than multimeric channels. As a result, the structures of the ion channel forms are unknown, but can be deduced from molecular modelling studies of assemblies of the monomeric structures [10,13,14]. In this study, an octameric channel structure for AAM has been produced based on the high resolution crystal structure of AAM-I. These modelling studies provide a basis for furthering our understanding of the details of structure/function relationships in cationic channels [15].

MATERIALS AND METHODS

A helical bundle model for the AAM channel was constructed using the SYBYL [16] molecular modelling package. The model was based on the AAM-I monomer 'A' crystal structure [9] (PDB ID code 1JOH). The 'A' monomer was chosen for use in these studies because its *N*-terminal segment was better ordered than that of the 'B' monomer. Conductance studies have suggested that an AAM channel contains $4n$ monomers [2]; eight monomers were therefore used to create the assembly, as a tetramer would not have a sufficiently large lumen, and a dodecamer would be expected to have a much larger single channel conductance than was observed. The octameric helical bundle channel model was generated by rotating the monomer through incremental 45° rotation about the *z*-axis (normal to the membrane surface). The eight monomers were then translated equidistantly along the *xy* axes to form a symmetrical pore. The model was optimized by varying the degree of translation, and by iteratively imposing small rotations on the initial monomer around the *z*-axis and by tilting the structure by rotation about the *x*-axis. Rotation and translation functions were carried out using the CCP4 program PDBSET [17]. The model was then subjected to 500 cycles of conjugate gradient least squares energy minimization in SYBYL [16]. In the model, the peptides are packed in parallel so that their *C*-termini are in close contact and the mouth of the channel opens out at the *N*-termini.

The CCP4 program AREAIMOL was used to calculate the accessible surface areas [18] of both the isolated monomers and the monomers in the channel assembly. The electrostatic surfaces were calculated in the program GRASP [19]. The program HOLE [20] was used to define the dimensions of the model channel lumen and also the lumens of the KcsA [21] (PDB code 1BL8) and MthK [22] (PDB code 1LNQ) potassium channel crystal structures.

RESULTS AND DISCUSSION

Modelling of the AAM Channel

One concern in using crystal structures of monomers for modelling studies, especially for relatively small molecules, is whether the structure in a crystal is substantially influenced either by the solvent present or by crystal packing forces. In the case of AAM-I, its structure has been determined in crystals formed from two solvents of very different polarity (methanol [9] and octanol [11]) and in two different crystal packing motifs, thereby enabling these influences to be investigated. The root mean square deviation (rmsd) between the backbone atoms in the two forms was only ~0.25 Å [23] and the central bend angles differed by only 3° [24]. In other words, the structures are virtually identical and thus any influence of environment on the AAM structure appears to be negligible, lending credence to channel models derived from these structures.

In the AAM monomer there are three imino acids (Hyp or Pro), which are incapable of forming intra-helical hydrogen bonds due to the missing amino hydrogens normally present in amino acids. This means that the carbonyls of residues separated

from the imino acids in the sequence by 3 or 4 residues have no hydrogen bonding partners. These carbonyl oxygens are thus free to interact with ions in the hydrophilic channel lumen. Importantly, the unpaired carbonyls of residues Gly6 and Leu7 are present on the same helical face as the hydrophilic Gln11 sidechains and are expected to line the lumen of the pore, thereby determining the orientation of the monomers with respect to the channel periphery in the model structure. In addition, these carbonyls form the end of the *N*-terminal alpha-helix which extends from residues 1–7; the helix dipole effect is therefore expected to result in a somewhat negative surface potential in this region [25].

The model channel structure consists of eight transmembrane helices, which form a parallel helical bundle (Figure 2a,b) with a hydrophobic exterior in contact with lipid and a hydrophilic lumen. Electrostatic calculations on the model suggest that the interior of the channel will have a moderately negative surface potential, which is consistent with its observed cation conductance. A preliminary model of AAM was published previously [24], but the model discussed in this paper, while generally similar in features, is more detailed, energetically refined, and has better stereochemistry.

In forming the octameric assembly, the total accessible surface area decreases by ~24% from that found in the isolated monomers, suggesting hydrophobic interactions will play a significant role in the channel formation and stability. Inter-helical hydrogen bonds are present between the Gln11 sidechains of adjacent monomers. The resulting hydrogen bonded ring (Figure 2b) could provide the dual function of stabilizing the helical bundle and producing a stretch of polarizable environment at the centre of the pore, which could lower the energy barrier for passage of hydrated or partially hydrated ions. The presence of a hydrogen bonded ring between the glutamine sidechains has been proposed for other peptaibol channel models, including alamethicin [10] and trichotoxin [13]. This ring imposes structural constraints on the model, resulting in a channel which is tightly packed around the *C*-terminal helices but splayed towards the *N*-termini. Calculations on the channel lumen dimensions show that this funnel-shaped pore is rather large: the minimum diameter of 9 Å occurs near Gln11, and then the lumen fans out to over 20 Å at the *N*-terminal end.

The AAM sequence has phenylalanine analogues at both termini; hence both ends of the structure are aromatic in character. When placed in a lipid

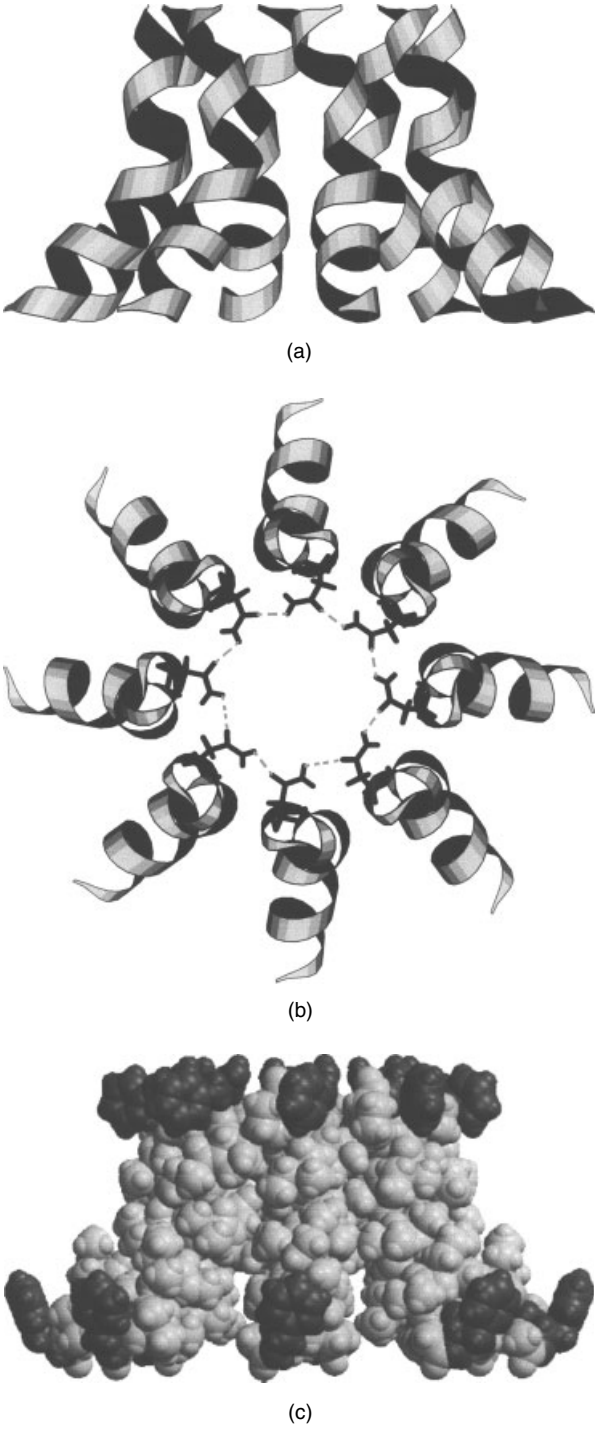

Figure 2 The structure of the AAM model channel depicted as (a) a helical bundle (side view), and (b) a helical bundle (top view) with Gln11 sidechains shown as solid sticks and the hydrogen bonds shown as dotted lines, and (c) a space-filling model (side view) showing the locations of the aromatic residues (shaded). These figures were created using MOLSCRIPT [30] and Raster3D [31].

bilayer, these residues would be located at the bilayer interface between the hydrophobic lipid fatty acid chains and the hydrophilic lipid head groups (Figure 2c). This is a structural feature seen for many membrane proteins [26] and is believed to be involved in stabilization of the transmembrane nature of the helices.

COMPARISON OF THE AAM CHANNEL MODEL WITH THE KCSA AND MTHK POTASSIUM CHANNEL CRYSTAL STRUCTURES

The first biological channel whose x-ray structure was determined was that of the KcsA potassium channel from *Streptomyces lividans* [21]. In many ways the KcsA channel crystal structure and the AAM channel model are similar and thus it is instructive to compare their structural and functional features. The active form of the potassium channel is a tetramer, with each of its monomers consisting of two transmembrane helices and a P region. Channels are produced by the association of monomers into a bundle of eight transmembrane helices, two of which are contributed by each monomer. This is similar to the AAM bundle, except that in the latter case, one helix is contributed by each of eight monomers (Figure 3a,b). Thus although the molecularities of the potassium channel and AAM are different (4 monomers vs 8 monomers), the number of transmembrane segments is the same for both types of channels.

The potassium channel has a narrow selectivity filter where the cations are specifically bound. The selectivity filter spans approximately one-third of the length of the internal pore, starting at the extracellular surface. Two cation binding sites separated by ~7.5 Å are formed from a region of the sequence consisting of alternating glycine residues. The sequence motif, TVGYG, is highly conserved amongst a wide range of homologous K^+ channels and can confer a >10 000 fold selectively for K^+ over the smaller Na^+ ion [21]. The stereochemical properties of the glycines enable the polypeptide backbone to adopt a conformation such that the lumen of KcsA is lined by several backbone carbonyl groups. A cation-binding region lined by backbone carbonyls is also a feature of the AAM channel model, although in this case the exposed carbonyls are accomplished in a different way (i.e. via imino acids).

At the centre of the KcsA pore is a water-filled cavity approximately 12 Å in length and 10 Å at its maximum width. The energy barrier for an ion to pass through a membrane channel generally has a

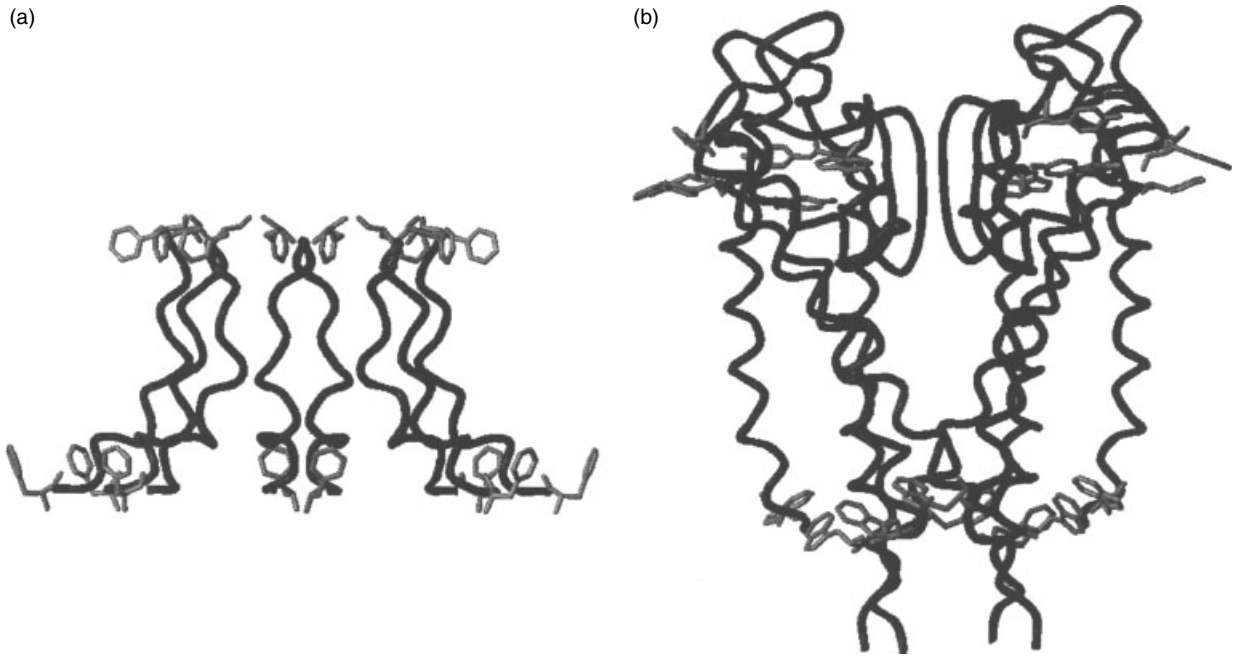

Figure 3 Comparison of (a) the AAM channel model and (b) the KcsA crystal structure. Backbones are depicted as dark 'worms' and the locations of the aromatic side chains are indicated in light grey stick mode. These figures were created in SYBYL [16].

maximum at the membrane centre [27]. The water-filled cavity in KcsA provides a polarizable medium to lower the energy barrier at the centre of the membrane. In the AAM channel model, the ring of Gln11 sidechains near the pore centre provides this function. In addition, the KcsA channel has the ends of helix dipoles pointing towards the central cavity, which aid in lowering the electrostatic barrier, a feature also found in the AAM model channel.

As observed for AAM, the aromatic amino acids of the potassium channel (particularly the tryptophans) are clustered at the membrane interface (Figure 3a,b). They tend to be oriented parallel to the membrane surface and have been suggested to produce an in-plane stabilization framework. In AAM, the phenylalanine rings are aligned closer to the membrane perpendicular.

The KcsA crystal structure appears to represent a closed conformation of the potassium channel, with a pore that narrows to a diameter of 4 Å at the intracellular end. The homologous MthK potassium channel [22] from *Methanobacterium thermoautotrophicum*, however, appears to be of the open conformation. It shares structural features with the KcsA channel, including a pore formed from eight transmembrane helices, a narrow selectivity filter based on the TVGYG motif and a large water-filled central cavity defined by the surrounding pore helices whose dipoles point inwards. However, the MthK channel was crystallized in its open conformation due to the presence of an intracellular calcium-gated domain, which is believed to exert a force on the pore inner helices, causing them to bend around a central glycine residue by an angle of ~30° [28]. This alters the helices from the straight conformation found in the closed channel to a conformation where the helices splay outwards, forming a large funnel shape at the intracellular end, similar to the AAM model (Figure 4a,b). This suggests that the AAM model may also be of an open channel conformation.

One difference, however, between the open and closed potassium channels and the AAM model is that potassium channel gating is related to bending of the pore inner helices, whereas peptaibol channel closure is believed to be related to monomer disassociation from the barrel-stave configuration. However, helical bending may play a role in voltage-gated channel insertion and assembly of AAM.

CONCLUSIONS

Despite the fact that AAM is a relatively small polypeptide and contains 'unusual' sequence features (such as non-standard amino acids and blocked termini), a channel model based on its crystal structure appears to have a three-dimensional structure which may be very similar to the types of structures found in larger and more complex biological channels.

AAM forms single-level channels, but only under very limited and specific conditions. In contrast, two other well-studied peptaibols, zervamicin and

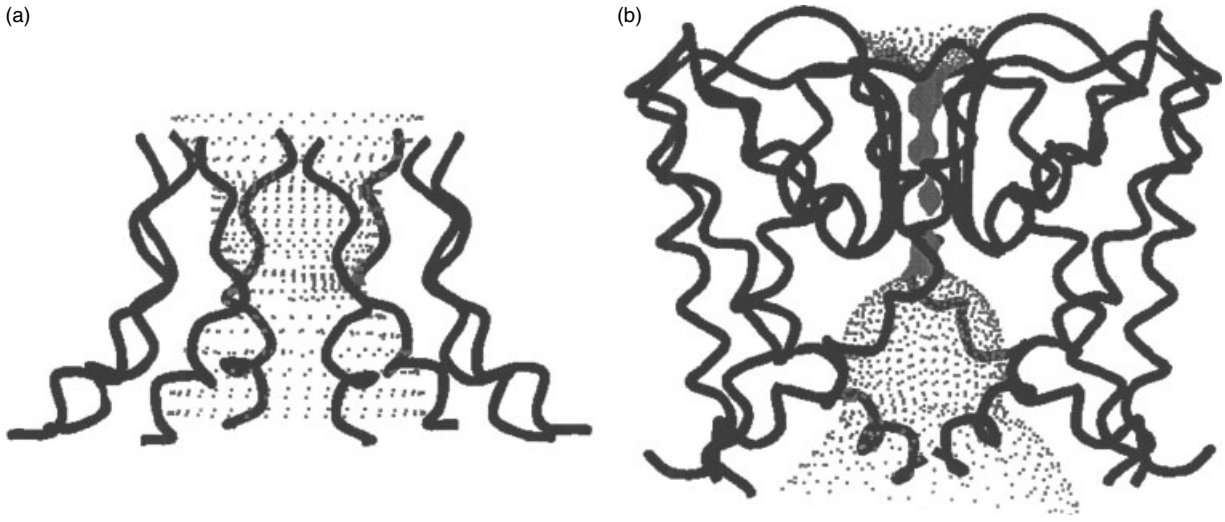

Figure 4 Comparison of the lumen dimensions of the (a) the AAM channel model and (b) the MthK crystal structure, using HOLE [20]. The polypeptide backbone structures are shown as dark worms, and the grey stippled regions indicate the extents of the channel lumens.

alamethicin, readily form voltage-activated channels under a wide range of conditions, but they are multi-level channels due to recruitment of different numbers of monomers per channel, a feature not detected in potassium channels. Hence, AAM may be a more suitable model for bacterial ion channels than these other peptaibols. Furthermore, similar single channel conductances have been observed for AAM, KcsA and MthK in planar bilayers: 90, 135 and 200 pS, respectively (electrolyte: 0.15–0.5 M KCl) [2,29,22] so they might be expected to have similar structural characteristics.

It was, therefore, instructive to compare structural features of the AAM model channel with the crystal structures of the KcsA and MthK potassium channels. Like AAM, these channels have a central lumen formed from the association of eight transmembrane helices. They do differ, however, in that in the AAM channels, the helices are all parallel, while in the potassium channels, half of the helices lie in an anti-parallel orientation. A water-filled cavity lowers the energy barrier to ion conductance mid-pore in the potassium channels, while the ring of hydrogen-bonds between side chains of Gln11 performs this function in the AAM channel. Also, the potassium channels and AAM channel have backbone carbonyl oxygens pointing to the pore centre to further stabilize ions mid-pore. The open MthK channel has a very wide lumen (>20 Å) at the intracellular surface, forming a funnel-shaped pore, a feature also found in the AAM channel.

The peptaibol family, consisting of relatively small, simple molecules, with a large number of naturally occurring 'mutants', provides a rich source of material for biophysical investigations [7,15], and the high resolution structures of these molecules could yet yield novel insights into ion conduction and channel stability. Since AAM forms channels in a moderately voltage-dependent manner, it could permit the investigation of relationships between polypeptide structural elements and voltage sensing. Furthermore, the small size of the peptaibol channels make them particularly suitable candidates for molecular simulation calculations and computational modelling of ion binding and transport processes [14].

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