

*Hypothesis*

# A helix dipole model for alamethicin and related transmembrane channels

M.K. Mathew and P. Balaram<sup>+</sup>*Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India*

Received 6 April 1983

A molecular model is proposed for the transmembrane channels formed by alamethicin and related polypeptides. The channels consist of an aggregate of rod-like helical polypeptides with a central aqueous core of ordered water. The helix dipole moment is considered to be the major factor modulating channel size, selectivity and field-dependent transitions.

<i>Membrane channel</i>	<i>Alamethicin</i>	<i>Helix dipole</i>	<i>Amphipathic helix</i>	<i>Membrane transport</i>
		<i>Peptide aggregation</i>		

## 1. INTRODUCTION

A number of polypeptides have been demonstrated to affect the permeability properties of membranes by forming channels through them. Sequences of a family of such peptides [1–6], rich in  $\alpha$ -aminoisobutyric acid (Aib) are shown in fig.1. Structural investigations of natural [7–10] and synthetic [11,12] antibiotics, fragments and model peptides [11,13] have demonstrated that the channel formers are largely helical, with  $3_{10}$  and  $\alpha$ -helical structures being suggested. Structure–activity correlations have demonstrated that the polar nature of the C-terminus is not essential for membrane activity [11,13,14]. In fact, a 13-residue N-terminal fragment of alamethicin has been shown to be active in ion transport [15] and uncoupling oxidative phosphorylation in mitochondria [16]. Hence, channel-forming activity lies in the helical segment of the peptides. Here, we present a model for peptide transmembrane channels as a working hypothesis.

## 2. AMPHIPATHIC HELICES AND AGGREGATION

Inspection of the sequences of the antibiotics reveals that they can form amphipathic helices; i.e., helices wherein the polar residues are largely clustered on one face leaving the others apolar [17,18]. This is illustrated for an  $\alpha$ -helical arrangement of zervamicin in fig.2A. Aggregation of such helices can expose either polar faces suitable for aqueous phase aggregates stabilized by hydrophobic interactions, or apolar faces for membrane phase aggregation (see fig.3) by rotation around the axes of individual helices. Such lipid phase aggregates have been suggested for delta lysin [18], suzukacillin [19] and the 7 helices of bacteriorhodopsin [20].

The helical structures taken up by these peptides are too narrow to allow the passage of ions through the interior of individual helices [21]. Hence, channel formation requires the aggregation of peptide helices, forming an interstitial channel (fig.3). Experimental support for this conclusion comes from the high power dependence (6th or 9th) of conductance on peptide concentration

<sup>+</sup> To whom correspondence should be addressed

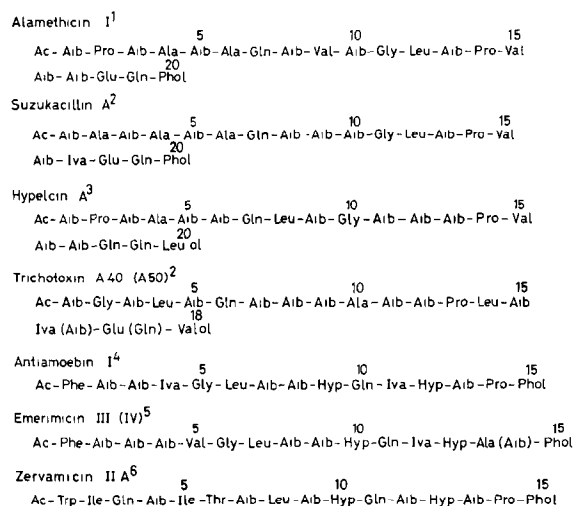


Fig.1. Sequences of some Aib containing antibiotics. Channel formation in planar membranes has been studied for alamethicin, suzukacillin, trichotoxin and zervamicin while hypelcin uncouples oxidative phosphorylation.

[22,23]. Further, aggregation has been observed in aqueous [9,24–26] and organic [19,27,28] solvents. If the conducting channel is an aggregate it follows that its lifetime will depend critically on the monomer–monomer interactions that stabilize the aggregate in the membrane phase. These include interchain hydrogen bonding and dipole–dipole interactions.

Analysis of the sequences reveals that the shorter antibiotics have residues (Thr, Hyp) with side

chains capable of hydrogen bonding while a Gln residue is conserved in all the peptides. This residue has been shown to stabilize aggregates of suzukacillin fragments in  $CHCl_3$  [19], and a crystal structure of alamethicin [10] indicates that it is involved in intermolecular hydrogen bonding, linking 2 of the 3 peptide molecules in the asymmetric unit. Lack of this residue drastically reduces lifetime of pores, which form none-the-less [29]. Thus, hydrogen bonding by sidechain NH or OH of one helix to backbone or sidechain carbonyls of an adjacent helix is likely to stabilize membrane aggregates and consequently lengthen channel lifetimes.

### 3. HELIX MACRO-DIPOLES

The other major stabilizing force is dipole–dipole interaction between peptide helices. Fig.2B shows the permanent dipole of the peptide unit and its near-parallel arrangement in helices to generate a large net dipole moment amounting to 3.5 D per 1.5 Å of an  $\alpha$ -helix [30,31]. The dipole moment of the alamethicin monomer has been reported to be 40–80 D, depending on the conditions of measurement [27,28], that for the aggregate in dioxane being anomalously low [27] indicative of antiparallel orientations of the constituent helices. Energies of interaction between helix dipoles have been estimated following the method in [31], assuming a dipole moment of 65 D after [32]. The difference in energies between the D and E states shown in fig.3 is  $\sim 60$  kcal.(mol aggregate) $^{-1}$  in the absence of an electric field. Hence the aqueous phase aggregate is likely to be a 'minimum dipole' aggregate. However, fields of the order of  $10^5$  V.cm $^{-1}$  in the membrane could populate high dipole states.

A probable sequence of events is shown in fig.3: aqueous phase aggregation exposing the polar faces of the helices; insertion into membranes with concomitant rotation about helix axes to present a hydrophobic exterior and facilitate intermolecular hydrogen bonding forming a 'closed pore'; voltage induced ejection of the 'core piece' in the aggregates (which is not hydrogen-bonded to its neighbours) thereby creating an open channel. The voltage dependence of alamethicin conductance has been ascribed to an increase in the number of open channels with increasing voltage [33,34]. The

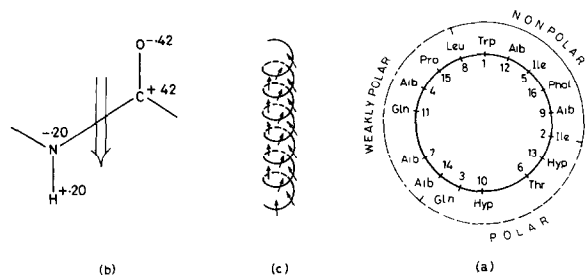


Fig.2. (A) Zervamicin IIA in an  $\alpha$ -helical arrangement viewed down the helix axis. Note the segregation of the residues into non polar, weakly polar and polar faces. (B) Excess charge distribution in a peptide unit showing the resultant dipole moment (from [30]). (C) Schematic representation of the near-parallel orientations of peptide dipoles in a helix.

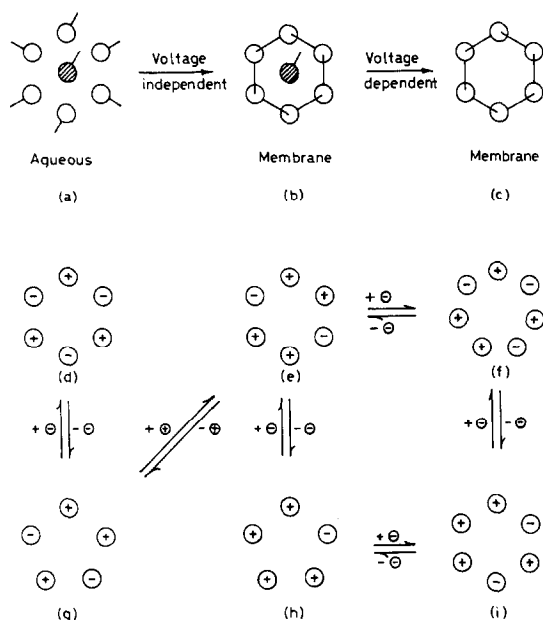


Fig.3. Schematic representation of the aggregation of peptide helices viewed in cross-section. + and - refer to opposite orientations of the helix dipole. Bar indicates the polar face of the helix (see fig.2A) and represents side chains capable of hydrogen bond formation. (A) Aqueous phase aggregate with polar face exposed to solvent; 'core piece' is hatched. (B) Membrane phase aggregate. Note rotation of helices to allow interchain hydrogen bonding and exposure of the apolar face. The 'core piece' is not hydrogen bonded and can be ejected by an applied potential to generate an open channel (C). (D-I) Schematic representation of pore state transitions. Equilibria shown do not represent actual pathways.

field experienced by an ion in the channel would be a resultant of the applied electric field, membrane surface charge effects and the field due to the helix dipoles. Hence, variations in the dipole states of the channels as depicted in fig.3 would be observed as variations in the conductance of the channel. Changes in the number of monomers making up a channel (fig.3) would also have this effect and could explain the observation that different conductance states of alamethicin have different channel diameters as estimated by a sieving technique [35].

The ion selectivity of these channels may be modulated by alterations in the aggregate size and dipole state which, in turn, alter the structures of the aqueous matrix. Such a channel should

transport protons efficiently, as in ice [36]. Their high efficiency as uncouplers of oxidative phosphorylation [16,37] support this contention.

Earlier models for the channel-forming activity of these peptides postulated that an applied voltage was obligatory for the insertion of the peptides into a membrane [34,38]. The dipole moment, when considered, was implicated in this step [34]. It has since been shown that no applied potential is required for the insertion of alamethicin into lipid membranes [39,40]. A more detailed model based on a crystal structure of alamethicin has been proposed, which postulates aggregates with Gln(7) sidechains protruding into the lumen [10]. The dipole moment of the sidechain and the applied potential determine the orientation of the Gln carboxamide group, which in turn modulates conductivity. This model ascribes an important role to the Gln sidechain in interacting with hydrated cations and attempts to build up a hydrophilic channel interior. It may be noted that the packing of molecules in the crystal does not provide any support for such an arrangement. The model also does not attempt to rationalize considerable structure-activity data, indicating that Gln(7) is not essential for the formation of voltage sensitive channels [42]. The crystal structure [10], however, confirms that the peptides take up helical structures and also detects intermolecular hydrogen bonding mediated by Gln(7), in support of this hypothesis. During preparation of this manuscript, the importance of helix dipoles in modulating membrane channel functions has also been recognized [43]. The model proposed here differs significantly in detail from [43], in that transmembrane 'flip-flop' is not necessary for imparting voltage sensitivity. Furthermore, helix amphipathicity and its influence on aggregation modes are explicitly considered here.

#### 4. CONCLUSION

The model proposed is intended to stimulate further experimental approaches to the study of molecular mechanisms involved in the ionophoric activity of these channel formers. The model ascribes a largely structural role for the cylindrical peptide aggregate in providing a scaffolding for supporting large aqueous columns, which serve to enhance the ion permeabilities of lipid bilayers.

The pores are postulated to be stabilized by dipole-dipole interactions and inter-chain hydrogen bonding and thus amenable to experimental verification. Deamidation of the central Gln should lead to significantly shorter lifetimes due to loss of hydrogen bond forming ability and charge repulsion; substitution with an apolar residue should have a less pronounced effect though both analogs should retain channel forming activity. The precise sequence of residues is not expected to be critical as long as the peptide is long enough (15–20 residues); constrained to adopt amphipathic  $\alpha$ - of  $3_{10}$  helical conformations; and is capable of forming intermolecular hydrogen bonds deep within the bilayer so as to stabilize the channel aggregate.

Preliminary studies indicate that analogs of suzukacillin fragments with Ala substituted for the central Gln are capable of forming transmembrane channels (unpublished). Reports of the activity of other channel analogs [13,29] and some synthetic polypeptides [29,41] are consistent with these predictions.

## ACKNOWLEDGEMENTS

The authors thank Professor Gunther Jung for providing preprints of his publications. Research in this area in this laboratory has been supported by the Department of Science and Technology, Government of India.

## REFERENCES

- [1] Pandey, R.C., Carter Cook, J. jr and Rinehart, K.L. jr (1977) *J. Am. Chem. Soc.* 99, 8469–8483.
- [2] Jung, G., Bosch, R., Katz, E., Schmitt, H., Voges, K.-P. and Winter, W. (1983) *Biopolymers*, in press.
- [3] Fujita, T., Takaishi, Y. and Shimomoto, T. (1979) *J. Chem. Soc. Chem. Commun.* 413–414.
- [4] Pandey, R.C., Carter Cook, J. jr and Rinehart, K.L. jr (1977) *J. Am. Chem. Soc.* 99, 5205–5206.
- [5] Pandey, R.C., Meng, H., Carter Cook, J. jr and Rinehart, K.L. jr (1977) *J. Am. Chem. Soc.* 99, 5203–5205.
- [6] Rinehart, K.L. jr, Gaudioso, L.A., Moore, M.L., Pandey, R.C., Carter Cook, J. jr, Barber, M., Segdwick, D., Bordoli, R.S., Tyler, A.N. and Green, B.N. (1981) *J. Am. Chem. Soc.* 103, 6517–6520.
- [7] Jung, G., Konig, W.A., Leibfritz, D., Ooka, T., Janko, K. and Boheim, G. (1976) *Biochim. Biophys. Acta* 433, 164–181.
- [8] Irmscher, G., Bovermann, G., Boheim, G. and Jung, G. (1978) *Biochim. Biophys. Acta* 507, 470–484.
- [9] Jung, G., Dubischar, N. and Leibfritz, D. (1975) *Eur. J. Biochem.* 54, 395–409.
- [10] Fox, R.O. and Richards, F.M. (1982) *Nature* 300, 325–330.
- [11] Nagaraj, R. and Balaram, P. (1981) *Acc. Chem. Res.* 14, 356–362.
- [12] Gisin, B.F., Davis, D.G., Borowska, Z.K., Hall, J.E. and Kobayashi, S. (1981) *J. Am. Chem. Soc.* 103, 6373–6377.
- [13] Jung, G., Bruckner, H. and Schmitt, H. (1981) in: *Structure and Activity of Natural Peptides* (Voelter, W. and Weitzel, G. eds) Walter de Gruyter, Berlin.
- [14] Mathew, M.K. and Balaram, P. (1983) *Mol. Cell. Biochem.*, in press.
- [15] Nagaraj, R., Mathew, M.K. and Balaram, P. (1980) *FEBS Lett.* 121, 365–368.
- [16] Mathew, M.K., Nagaraj, R. and Balaram, P. (1981) *Biochem. Biophys. Res. Commun.* 98, 548–558.
- [17] Segrest, J.P. (1977) *Chem. Phys. Lip.* 18, 7–22.
- [18] Freer, J.H. and Birkbeck, T.H. (1982) *J. Theor. Biol.* 94, 535–540.
- [19] Iqbal, M. and Balaram, P. (1981) *Biochemistry* 20, 7278–7284.
- [20] Engelman, D.M. and Zaccari, G. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5894–5898.
- [21] Van Duijnen, P.Th. and Thole, B.T. (1981) *Chem. Phys. Lett.* 83, 129–133.
- [22] Mueller, P. and Rudin, D.O. (1968) *Nature* 217, 713–719.
- [23] Balasubramanian, T.M., Kendrick, N.C.E., Taylor, M., Marshall, G.R., Hall, J.E., Vodyanoy, I. and Reusser, F. (1981) *J. Am. Chem. Soc.* 103, 6127–6132.
- [24] Mc Mullen, A.I. and Stirrup, J.A. (1971) *Biochim. Biophys. Acta* 241, 807–814.
- [25] Mathew, M.K., Nagaraj, R. and Balaram, P. (1981) *Biochim. Biophys. Acta* 649, 336–342.
- [26] Mathew, M.K., Nagaraj, R. and Balaram, P. (1982) *J. Biol. Chem.* 257, 2170–2176.
- [27] Schwarz, G. and Savko, P. (1982) *Biophys. J.* 39, 211–219.
- [28] Yantorno, R., Takashima, S. and Mueller, P. (1982) *Biophys. J.* 38, 105–110.

- [29] Jung, G., Bruckner, H., Oekonomopulos, R., Boheim, G., Breitmaier, E. and König, W.A. (1979) in: *Peptides, Structure and Biological Function*. 6th Am. Peptide Symp. (Gross, E. and Meienhoffer, J. eds) pp.647-654, Pierce Chemicals, Rockford IL.
- [30] Wada, A. (1976) *Adv. Biophys.* 9, 1-63.
- [31] Hol, W.G.J., Halie, L.M. and Sander, C. (1981) *Nature* 294, 532-536.
- [32] Yantorno, R.E., Takashima, S. and Mueller, P. (1977) *Biophys. J.* 17, 87a.
- [33] Eisenberg, M., Hall, J.E. and Mead, C.A. (1973) *J. Membr. Biol.* 14, 143-176.
- [34] Gordon, L.G.M. and Haydon, D.A. (1975) *Phil. Trans. Roy. Soc. Ser. B* 270, 433-447.
- [35] Hanke, W. and Boheim, G. (1980) *Biochim. Biophys. Acta* 596, 456-462.
- [36] Williams, R.J.P. (1975) in: *Electron Transfer Chains and Oxidative Phosphorylation* (Quagliariello, E. et al. eds) pp.417-422, Elsevier, Amsterdam, New York.
- [37] Takaishi, Y., Terada, H. and Fujita, T. (1980) *Experientia* 36, 550-552.
- [38] Boheim, G. and Kolb, H.-A. (1978) *J. Membr. Biol.* 38, 99-150.
- [39] Latorre, R., Miller, C.G. and Quay, S. (1981) *Biophys. J.* 36, 803-809.
- [40] McIntosh, T.J., Ting-Beall, H.P. and Zampighi, G. (1982) *Biochim. Biophys. Acta* 685, 51-60.
- [41] Heitz, F. and Spach, G. (1982) *Biochem. Biophys. Res. Commun.* 105, 179-185.
- [42] Jung, G., Katz, E., Schmitt, H., Voges, K.P., Menestrina, G. and Boheim, G. (1983) in: *Physical Chemistry of Transmembrane Motions* (Trojanowsky, C. ed) Elsevier, Amsterdam, New York, in press.
- [43] Boheim, G., Hanke, W. and Jung, G. (1983) *Biophys. Struct. Mech.* 9, 181-191.