Ion channel formation by zervamicin-IIB

A molecular modelling study

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Abstract. Zervamicin-IIB (Zrv-IIB) is a 16 residue peptai-
bol which forms voltage-activated, multiple conductance
level channels in planar lipid bilayers. A molecular model
of Zrv-IIB channels is presented. The structure of
monomeric Zrv-IIB is based upon the crystal structure of
Zervamicin-Leu. The helical backbone is kinked by a
hydroxyproline residue at position 10. Zrv-IIB channels
are modelled as helix bundles of from 4 to 8 parallel
helices surrounding a central pore. The monomers are
packed with their C-terminal helical segments in close
contact, and the bundles are stabilized by hydrogen
bonds between glutamine 11 and hydroxyproline 10 of
adjacent helices. Interaction energy profiles for move-
ment of three different probes species (K+, Cl- and wa-
ter) through the central pore are analyzed. The confor-
mations of: (a) the sidechain of glutamine 3; (b) the
hydroxyl group of hydroxyproline 10; and (c) the C-ter-
minal hydroxyl group are "optimized" in order to maxi-
mize favourable interactions between the channel and the
probes, resulting in favourable interaction energy profiles
for all three. This suggests that conformational flexibility
of polar sidechains enables the channel lining to mimic an
aqueous environment.

Key words: Ion channel – Peptaibol – Molecular mod-
elling – Channel-forming peptide

Introduction

The past decade has witnessed a substantial increase in
interest in the molecular properties of receptor-gated
ion channels, concomitant with an expansion of the se-
quence database for these multi-subunit, trans-mem-
brane proteins. A low resolution image of the structure of
the prototypic member of this superfamily, the nicotinic
acetylcholine receptor, has been obtained by e.g. Toyo-
shima and Unwin (1990) using cryoelectron microscopy.

Unfortunately, it has not proved possible to obtain atom-
ic resolution structural information. However, insights
into the molecular nature of the central pore region of
channel proteins have been gained from studying simple
model systems, namely channel forming peptides (CFPs; Sansom 1991).

CFPs are short (ca. 20 residues) hydrophobic peptides
which adopt an α-helical conformation in the presence of
lipid bilayers. Trans-membrane voltages induce them to
form ion channels in bilayers, of comparable functional
properties (conductance, ion selectivity etc.) to those of
channel proteins. Channels are formed by a process of
self-assembly within the plane of the bilayer which gener-
ates bundles of parallel trans-membrane helices. These
helices surround a central pore, which is lined by hy-
drophilic sidechains, thus permitting permeation of se-
lected ions. One reason for studying CFPs is that it is
possible to relate their functional (i.e. electrical) properties
to structural (NMR and/or X-ray diffraction) information
in order to develop molecular models of channel struc-
ture.

The peptaibols are a family of channel-forming pep-
tides which contain a high percentage of the helix-pro-
moting residue α-aminoisobutyric acid (Aib). Each pepta-
bol molecule contains at least one proline residue, and
the C-terminus is usually an α-amino alcohol e.g. phenyl-
alaninol. The most intensively studied peptaibol is alame-
thicin (Alm; Mathew and Balaram 1983; Hall et al. 1984;
Boheim et al. 1987), a 20 residue CFP. More recently,
investigations have been extended to the zervamicins
(Zrv), a family of 16 residue peptaibols isolated from
Emericellopsis salmosynnemata (Rinehart et al. 1981;
Krishna et al. 1990).

The channel-forming properties of zervamicin-IIB
(Zrv-IIB) have been studied in some detail, (Agarwalla
et al. 1992; Balaram et al. 1992). Channels are believed to
be formed by parallel bundles of Zrv-IIB helices sur-
rounding a central ion-permeable pore. Zrv-IIB has been
shown to form multiple conductance level channels.
The different conductance levels correspond to different
numbers of monomers per bundle (N). The sequence of
Zrv-IIB is:

Ac-W-I-Q-J-I-T-U-L-U-O-Q-U-O-U-P-F-OH

where U = \(\varepsilon\)-aminoisobutyric acid, O = hydroxyproline, 
J = (R)-isovaline and F-OH is the C-terminal phenylalaninol. The crystal structure of a closely related species, Zrv-Leu (which differs from Zrv-IIB only in that residue 1 is leucine rather than tryptophan; Karle et al. 1991), reveals the molecule to be largely helical, with a central kink introduced by the hydroxyproline at position 10. Previous modelling studies have suggested that such proline-induced kinks may provide cation binding sites in ion channels (Sansom 1992a). In this paper the structure of Zrv-Leu is used to model the structure of Zrv-IIB, which in turn is used to develop molecular models of Zrv-IIB channels. Zrv-IIB is an attractive candidate for modelling studies as it does not contain any ionizable sidechains. This avoids the problems of calculation of local pK\(_a\)s when such sidechains are in relatively close proximity within intramembraneous helix bundles. A preliminary account of some of this work has appeared in abstract form (Sansom 1992c).

**Methods**

**Helix bundles**

All modelling was performed using QUANTA 3.2 (Polygen, Waltham, MA), run on a Silicon Graphics (Mountain View, CA) Indigo workstation. Molecular mechanics calculations were carried out using CHARMM (Brooks et al. 1983). Molecular structures were drawn using MolScript (Kraulis 1991). All auxiliary programs were written in Fortran77.

Helix bundles are generated using bndlg, as described in previous publications (Sansom et al. 1991; Sansom 1992a, b). Bundles are aligned such that the central pore axis is coincident with the z axis. Looking down z such that the N-termini are towards the viewer, the helices are named A, B, ... in an anticlockwise manner. Thus e.g. O\(_7\): 10 (A) refers to the O\(_7\) atom of residue 10 in helix A.

**Hydrophilic surfaces**

In constructing helix bundle models the first step is definition of the hydrophilic face of an amphipathic helix. This is done via empirical energy function calculation of the interaction of a water molecule with the surface of the helix. The oxygen atom of a water molecule is placed at successive positions, (\(z, r, \phi\)), on a cylindrical polar grid. The \(z\)-axis of the grid is coincident with the helix axis. For each position of the water molecule, an energy minimization is performed in which peptide atoms and water oxygen atom are fixed whereas the water hydrogen atoms are free to move, thus generating an optimum orientation of the water molecule. The peptide-water interaction energy is then evaluated as the sum of a van der Waals and an electrostatic term, generating an array containing the interaction energy at each grid point, \(E(z, r, \phi)\). For each value of \((z, \phi)\) the minimum value of \(E\) with respect to \(r\) is selected. The resultant \(E_{\text{min}}(z, \phi)\) array is displayed as a contour plot, thus revealing the hydrophilic surface of the helix. A secondary plot is obtained by averaging \(E_{\text{min}}\) over \(z\) to yield \(\langle E\rangle\). The minimum in a graph of \(\langle E\rangle\) vs. \(\phi\) thus defines the centre of the hydrophilic face of the helix, which is in turn used to determine orientation of the helix within a bundle. A more detailed exposition of this procedure and of its application to non-peptidol CFPs is the subject of a forthcoming paper (Kerr and Sansom 1992).

**Interaction energy profiles**

Channel-ion interaction energy profiles were evaluated as in previous papers (Sansom et al. 1991; Sansom 1992a, b). The aim of these calculations is to probe for ion-lingading sites within the pore defined by a helix bundle. They do not estimate permeation profiles for ions moving through the pore, as the models do not include water and so fail to take into account solvation/desolvation energies of the ion. However, the results are of value in that they enable one to focus on possible ion-protein interactions within the bilayer region.

In these calculations, the channel model is treated as a rigid body. Polar hydrogen atoms are explicitly included, whereas non-polar hydrogens are included via an extended atom representation. The ion probe is placed at points along the \(z\)-axis. The empirical energy of interaction between ion and channel is evaluated as the sum of a van der Waals and an electrostatic term. In calculating the electrostatic energy a distance dependent dielectric is employed in order to mimic the effect of solvent-screening on channel-ion interactions at moderate separations. This method of probing the channel for potential liganding sites is related to the GRID program of Goodford (1985) and to the method of Furois-Corbin and Pullman (1986).

Ion-channel interaction energies are evaluated as the difference between the energy with the ion at a given point and the energy when ion and channel are separated by a considerable distance (typically \(\geq 5.0 \text{ nm}\)). Energy profiles are generated by translating the ion along the pore (i.e. \(z\) axis), the interaction energy being evaluated as a function of \(z\).

A similar method has been used to probe channel-water interactions. In these calculations the oxygen atom of a water molecule is placed at successive positions along \(z\), and the optimum orientation determined in the same manner as described above for hydrophilic surface evaluations. The channel-water interaction energy is evaluated as the difference between the energy with the water at a given value of \(z\) and at \(z \geq 5.0 \text{ nm}\).

**Results**

**Monomer structure**

The structure of monomeric Zrv-IIB was modelled using the crystal structure of Zrv-Leu (Karle et al. 1991). The leucine residue at position 1 was replaced by a tryptophan.