Structural model of a voltage-gated potassium channel based on spectroscopic data

P. I. Hark
Department of Biological Sciences, De Montfort University, The Gateway, Leicester, LE1 9BH, U.K.

Abstract
It is estimated that membrane proteins comprise as much as 30% of most genomes. Yet our knowledge of membrane-protein folding is still in its infancy. Consequently, there is a great need for developing approaches that can further advance our understanding of how peptides and proteins interact with membranes and thereby attain their folded structure. An approach that we have been exploring involves dissecting voltage-gated ion channels into simple peptide domains for the purpose of determining their structure in different media using physical techniques. We have synthesized peptides corresponding to the six membrane-spanning segments, as well as the pore domain, of the Shaker channel and characterized their secondary structures. From these studies we have developed a model for the transmembrane structure of the Shaker potassium channel that is constructed from α-helices. The hard structural data obtained from these studies lends support to the recent theoretical models of this channel protein that have been developed by others.

Introduction
Despite their importance, and the fact that they make up about 30% of most genomes, our understanding of membrane-protein structure remains rather poor. Such is the scale of our ignorance that high-resolution X-ray structures of about a dozen different membrane proteins are known, whereas such structures are available for hundreds of
water-soluble proteins. This disproportionately low quantity of structural data for membrane proteins can be attributed to their greater complexity compared with water-soluble proteins. More than anything else it is their existence in a hydrophobic, phospholipid bilayer membrane that makes their structural characterization notoriously difficult. There are few physical techniques that can be used to characterize membrane-protein structure in a lipid-bilayer environment. Extracting membrane proteins from this biomembrane environment, so that biophysical measurements can be carried out, can often lead to irreversible structural and functional changes.

The situation is not made any easier by the fact that many membrane proteins exist in low quantities in natural sources, and their purification and overexpression is much more difficult compared with water-soluble proteins. Despite these difficulties, progress in our understanding of membrane-protein structure continues to advance, through different avenues, albeit at a rather slow pace. The wealth of membrane-protein sequence data that is available has been effectively used for carrying out diverse structure-function studies primarily using molecular-biological and biochemical tools. Many important principles regarding membrane-protein folding and function have emerged from such studies. The major obstacle that has prevented utilization of such data for elucidating the molecular details of membrane-protein-mediated processes is the scarcity of hard structural data that can only be attained through application of physical techniques such as cryoscopic and spectroscopic methods.

**Approaches for structural characterization of voltage-gated ion channels**

The difficulties, hopes and challenges encountered in studying membrane-protein structure can be best illustrated using the example of ion channels. For some time the amino acid sequences of a number of voltage-gated ion channels have been known. The first sequence, that of a sodium channel, was reported back in 1984 [1]. Using these sequence data a three-dimensional structural model of the sodium channel was reported in 1986 [2]. Subsequently, the sequences of a number of different sodium as well as potassium and calcium ion channels were elucidated. The ability to clone ion channels spurred a series of mainly molecular biological, biochemical and electrophysiological studies. These studies attempted to relate the role of different amino acid residues, and hence structure, to the functional mechanism of ion channels.

Mutagenesis studies combined with electrophysiological measurements continued to play the major role in identifying structurally and functionally important segments of these ion channels. Data obtained from such studies were used to produce more detailed theoretical models of the structural organization of these ion channels [3]. A major breakthrough came in 1998 when the first crystal structure of an ion channel was reported [4]. It was a bacterial potassium channel that was available in sufficient quantities for crystallization and other biophysical studies. As yet, however, the structures of the more complex voltage-gated ion channels remain unknown. Very recent progress has been the low-resolution (19 Å) determination of the three-dimensional structure of a voltage-sensitive sodium channel from the eel *Electrophorus electricus* [5]. The structure was determined by helium-cooled cryo-electron microscopy and single-particle image analysis of the solubilized sodium channel. The channel was found to possess a bell-shaped outer surface, a square-shaped bottom and a spherical top. It is thought that homologous voltage-sensitive calcium and tetrameric potassium channels may possess a related structure.

The channel structures that have been structurally characterized so far, both the sodium channel and the bacterial potassium channel, were derived from sources in which they are available in large quantities. Hence, this made it possible to attempt crystallization trials and other biophysical measurements. Unfortunately, this is not readily possible for many other ion channels, including the Shaker and mammalian voltage-gated potassium channels. Nevertheless, this has not prevented attempts at characterization of the folding of such proteins in biomembranes. Besides theoretical prediction methods and mutagenesis studies, other approaches have been developed to gain some insights into the folding of ion channels. One approach involves dissecting membrane proteins into specific domains and subsequently reconstructing their structure on the basis of spectroscopic data. This was first shown to be useful through studies of bacteriorhodopsin in which peptide fragments corresponding to its transmembrane regions were separately refolded into lipid bilayers and subsequently brought together to yield a functional protein [6]. This led to the proposal of a so-called two-stage model of mem-
brane-protein folding which suggests that isolated transmembrane \( \alpha \)-helices form stably in the bilayer before coming together to form the fully functional protein. This is a particularly attractive approach for characterizing the structures of voltage-gated potassium ion channels, as studies can be carried out with synthetic peptides, bypassing the need for purified ion-channel protein from natural sources. It provides a method for investigating the role of protein–protein and protein–lipid interactions in the folding and assembly of voltage-gated ion channels. We embarked on a series of studies on synthetic peptides corresponding to functionally and/or structurally important segments of voltage-gated ion channels \([7,8]\). The first of these studies focused on the pore domain of the Shaker voltage-gated potassium channel \([7]\).

Subsequently, peptides corresponding to the transmembrane segments of the protein were synthesized and their conformation characterized in different media using diverse spectroscopic techniques.

**Structure of the six transmembrane segments**

From hydropathy analysis (see Figure 1), voltage-gated potassium channels have been predicted to have six transmembrane segments \((S1–S6)\). These segments are suggested to be helical since the transmembrane structure of the majority of the membrane proteins determined to date are helical.

Durell and Guy \([3]\) combined secondary structure prediction with available mutagenesis and electrophysiological data to propose three-dimensional models of the Shaker voltage-gated potassium channel. The reliability of such theoretical models, however, requires verification using hard structural data derived from physical techniques. We

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**Figure 1**

Transmembrane topology of the Shaker voltage-gated potassium channel

The extracellular region is depicted above the lipid membrane. Each subunit contains six transmembrane segments \((S1–S6)\) and a P loop between segments S5 and S6. (I) The P loop is depicted as a \( \beta \)-hairpin, as was initially proposed \([9–11]\). (II) The P loop is now depicted as partially helical. The suggestion of a helical structure is based on our spectroscopic characterization of a synthetic peptide corresponding to this segment \([7]\) as well as evidence based on theoretical modelling \([3]\) and the X-ray structure of a homologous domain of the bacterial potassium channel \([4]\).
chemically synthesized each of the six transmembrane segments (S1–S6) of the Shaker channel and reconstituted them into phospholipid vesicles. Secondary structures of the peptides were assessed using Fourier-transform IR (FTIR) spectroscopy, which is one of the techniques that can be used for conformational analysis of peptides and proteins in phospholipid membranes. These studies reveal that in a hydrophobic environment, reflected by phospholipid membranes, each of the six transmembrane domains of the Shaker channel adopt a predominantly α-helical structure. Since the X-ray structure of the Shaker channel is not known, these biophysical measurements provide direct evidence to suggest that the transmembrane segments are indeed helical.

The structure of the pore domain: α-helix or β-sheet?

Mutagenesis experiments on voltage-gated potassium channels have suggested that the ion-selective pore is comprised mostly of P (or H5) segments, a stretch of 21 contiguous residues (see Figure 1), located between transmembrane segments S5 and S6 (see [9–11]). The pore region was suggested to be in a β-hairpin structure (see Figure 1, panel I) [9–11]. This was rather surprising since transmembrane segments are generally considered to be helical. This deviation from the norm was an attempt to rationalize the results of mutagenesis data and the effect of ion-channel blockers, which suggest that the P segment spans the membrane, at least partially, twice [9–11]. Since a sequence of 21 residues is far too short to span the bilayer twice as an α-helix, a β-hairpin structure was suggested instead, as this conformation would fit the requirement of spanning the bilayer twice. Subsequently, a number of theoretical models for the structure of the pore region were reported (see for example [12,13]). The majority of these studies favour the original suggestion, based on rationalization of the mutagenesis data, that it is likely to be in a β-sheet conformation [12,13]. However, Durell and Guy [3] were to report the first prediction study that favoured a helical structure for the pore segment. The conflicting results of the prediction analysis can only be resolved through biophysical studies. We chemically synthesized the pore domain and characterized its structure in phospholipid membranes. The secondary structure of the peptide, determined using CD and FTIR spectroscopies, was predominantly helical [7]. Since the crystal structure of a Shaker channel is not known, it is not possible to be certain whether the pore domain, in the native channel, adopts a helical structure or not. However, the homologous domain in the X-ray crystal structure of the bacterial potassium channel has a partial helical structure with the distinct absence of any β-sheet structure [4]. Further support for our observation of a helical structure comes from the studies of Lu and Miller [14]. These authors introduced cysteine at each P-region position in a Shaker potassium channel. They identified residues projecting side chains into the pore through channel inhibition by a thiol-reactive potassium ion analogue, silver ion. The pattern of silver-ion reactivity was found to be more consistent with a helical periodicity and contradicted a β-barrel architecture for potassium-channel pores. Thus, the available experimental data support the conclusion reached earlier in studies utilizing peptide fragments corresponding to the pore region. This clearly demonstrates the usefulness of the peptide-dissection approach in understanding membrane-protein folding.

S4 voltage-sensor domain displays helix-coil conformational switching

Of the six transmembrane peptides synthesized, the S4 peptide was the most unusual. It was the only transmembrane peptide that was water-soluble. This was not entirely unexpected since it contains an unusually large number of charged residues for a region thought to span the lipid bilayer. However, the fact that the S4 peptide was water-soluble made it amenable to diverse biophysical studies. Its conformation was characterized in water and was surprisingly found to be largely random-coil [8]. However, in phospholipid membranes it changes its conformation, showing an increase in helical structure [8]. According to our FTIR analysis the extent of helicity in phospholipid membranes was dependent on both the charge of the lipid headgroups and the fluidity of the membrane. The helicity was greater in negatively charged phospholipid membranes, especially in the fluid phase of the membrane. Conformational changes in the S4 segment associated with ion-channel gating have recently been reported [15,16]. Although large-scale movement of the S4 has been observed, it is not clear if this also includes major changes in its secondary structure.

Studies carried out with isolated fragments of the S4, in the absence of voltage, are unlikely to truly reflect the environment encountered by this
region in the native channel. Nevertheless, these studies provide valuable information regarding the conformational characteristics of the S4 domain that can contribute towards building a better understanding of its role in the gating process. From our studies we find that the S4 peptide not only undergoes helix-coil transition but is also sensitive to the fluidity of the lipid bilayer and the charge on the lipid head groups. This highly sensitive nature of the S4 peptide may not be surprising considering that it stands alone, amongst the transmembrane segments of the voltage-gated ion channels, in being water-soluble and containing charged residues. Indeed, the sensitivity of the S4 peptide structure to variation in its surrounding environment can be considered a valuable asset for a region of the ion channel that is thought to act as a voltage sensor.

Model of the potassium channel based on spectroscopic data from synthetic peptides

The model of the voltage-gated potassium channel structure that we propose, based on spectroscopic studies of peptide domains corresponding to S1–S6 and the P domain of the Shaker potassium channel, is presented in Figure 1 (panel II). This model is in excellent agreement with the latest theoretical model of Guy and co-workers [3]. Whereas FTIR and CD spectroscopy have been valuable for providing information regarding the overall secondary structure of the peptides, they cannot provide the complete three-dimensional structure at the level of individual residues. For this one needs to carry out either X-ray crystallography or NMR spectroscopy. We are currently characterizing the structure of synthetic peptides corresponding to the Shaker channel using NMR spectroscopy in a bid to gain further insights into the detailed folding of the individual domains. Through this approach we hope to develop a complete three-dimensional picture of the Shaker potassium channel that can be compared with theoretical models developed by Durell and Guy [3], and can also be used to rationalize the wealth of mutagenesis and electrophysiological data.

I would like to dedicate this article to the memory of Professor Dennis Chapman FRS, in whose laboratory this project was initially started.

References


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