ELSEVIER

Toxicon 39 (2001) 739–748

TOXICON

www.elsevier.com/locate/toxicon

Review

Potassium channels: from scorpion venoms to high-resolution structure

M.L. Garcia[a,*], Ying-Duo Gao[b], O.B. McManus[a], G.J. Kaczorowski[a]

[a]*Department of Membrane Biochemistry and Biophysics, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Molecular Systems, Merck Research Laboratories, P.O. Box 2000, Rahway, NJ 07065, USA*

Received 8 June 2000; accepted 5 July 2000

1. Introduction

In 1998, the field of ion channel research entered a new era when the first, high-resolution crystal structure of one of these proteins was solved (Doyle et al., 1998). For the first time, it was possible to understand, at a molecular level, the mechanisms that control ion selectivity and conduction in potassium channels. The protein whose structure had been determined, the KcsA K^+ channel, is a two transmembrane spanning domain, potassium selective channel from *Streptomyces lividans* that gates in response to H^+ when reconstituted in artificial lipid bilayers (Cuello et al., 1998; Heginbotham et al., 1999). The physiologic role of the KcsA channel in *Streptomyces* is not well understood. The amino acid sequence of the KcsA channel is closely related to that of some eukaryotic, six-transmembrane spanning domain, potassium channels (Doyle et al., 1998). In particular, the sequence in the pore region, containing the K^+ channel signature sequence, is almost identical to that found in vertebrate voltage-gated K^+ channels. An exciting aspect of the KcsA channel study was the finding that a number of structural features predicted from functional studies with eukaryotic six-transmembrane domain voltage-gated K^+ channels were present in the determined structure of the prokaryotic channel. This provided compelling evidence that the overall architecture of K^+ channels may be conserved through evolution, and that the structure of the KcsA channel can be used as a paradigm to probe the properties of other K^+ channels.

The interpretation of structural features of the KcsA channel was greatly facilitated by a large amount of functional data accumulated in previous years with several eukaryotic K^+ channels. Two major factors have facilitated the efforts of the many research laboratories that have focused on the study of K^+ channels: (1) the extensive cloning and functional expression of these proteins; and (2) the existence of a large number of high affinity peptidyl inhibitors of these proteins, isolated from different scorpion and spider venoms (Tytgat et al., 1999). In fact, the use of peptidyl inhibitors derived from scorpion venoms provided the first indirect information concerning K^+ channel structure. For instance, both identification of the pore region of the channel (MacKinnon and Miller, 1989b), and determination of the tetrameric composition of K^+ channels (MacKinnon, 1991) were made possible with the use of scorpion toxins. In addition, these peptides have been invaluable tools for purifying channels from native tissues, as well as determining their subunit composition (Garcia-Calvo et al., 1994), and also for understanding the physiological role of specific channel proteins (Garcia et al., 1997). Clearly, scorpion toxins have played important, crucial roles in determining potassium channel structure and function. In this review, we will discuss how work with scorpion venom peptides have progressed from their initial discovery as K^+ channel blockers, to their prominent role in interpreting the structure of the KcsA channel, and to the role that they may play in the future. Other aspects of work with these inhibitors, such as those concerning their role in developing the molecular pharmacology of potassium channels have previously been discussed extensively (Garcia et al., 1997; Garcia and Kaczorowski, 1998), and, therefore, will not be reviewed here.

2. Potassium channel blocking peptides

All potassium channel blocking peptides that have been purified from scorpion venoms contain 30–40 amino acids with three or four disulfide bridges. Based on primary sequence homology, 12 sub-families containing 49 different peptides, termed α-KTX_{1-12}, have been described (Tytgat

* Corresponding author. Tel.: +1-732-594-7564; fax: +1-732-594-3925.

E-mail address: maria_garcia@merck.com (M.L. Garcia).

PII: S0041-0101(00)00214-2

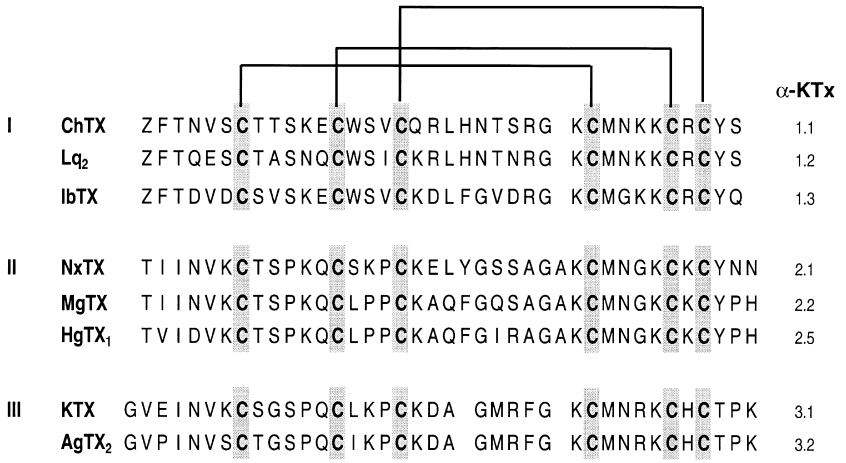

Fig. 1. Comparison of the amino acid sequences of charybdotoxin (ChTX; α-$KTX_{1.1}$), *L. quinquestriatus* toxin 2 (Lq_2; α-$KTX_{1.2}$), iberiotoxin (IbTX, α-$KTX_{1.3}$), noxiustoxin (NxTX; α-$KTX_{2.1}$), margatoxin (MgTX; α-$KTX_{2.2}$), hongotoxin1 ($HgTX_1$; α-$KTX_{2.5}$), kaliotoxin (KTX; α-$KTX_{3.1}$), and agitoxin2 ($AgTX_2$; α-$KTX_{3.2}$) (Tytgat et al., 1999). The sequences have been aligned with respect to the six cysteine residues which are highlighted. The position of the disulfide bonds is indicated. Peptides are grouped in subfamilies according to their sequence homologies.

et al., 1999). Out of these 49 peptides, only a restricted number have been studied in detail with respect to their interaction with K^+ channels. Noxiustoxin, α-$KTX_{2.1}$, purified from venom of the scorpion *Centruroides noxius* in 1982, was the first identified K^+ channel blocking peptide (Possani et al., 1982). Charybdotoxin (ChTX; α-$KTX_{1.1}$), identified in 1985 as a component of *Leiurus quinquestriatus* var. *hebraeus* venom, became the standard K^+ channel inhibitory peptide (Miller et al., 1985; Garcia et al., 1995). Subsequently, iberiotoxin (IbTX; α$KTX_{1.3}$) (Galvez et al., 1990), margatoxin (MgTX; α-$KTX_{2.2}$) (Garcia-Calvo et al., 1993), kaliotoxin (KTX; α-$KTX_{3.1}$) (Romi et al., 1993), and agitoxin-2 ($AgTX_2$; α-$KTX_{3.2}$) (Garcia et al., 1994) (Fig. 1) were found to interact with higher affinity and specificity at some types of K^+ channels, and these peptides became prototype inhibitors of their respective target channels.

The three-dimensional structure of a number of these peptides has been solved in solution by NMR techniques (Bontems et al., 1991, 1992; Johnson and Sugg, 1992; Fernandez et al., 1994; Johnson et al., 1994; Dauplais et al., 1995; Krezel et al., 1995). All of these peptides adopt a backbone conformation consisting of three anti-parallel beta strands linked to an α-helical region by disulfide bonds (Fig. 2). This folding pattern causes the molecule to have a very compact structure where all residues, except for the cysteines, are exposed to solvent. The best characterized peptides (ChTX, IbTX and $AgTX_2$) interact with members of the K_v1 family of voltage-gated K^+ channels and/or calcium-activated potassium channels (Garcia et al., 1997). Mechanistic studies carried out in different laboratories clearly identify these peptides as pore blockers. A good body of evidence indicates that all the peptides bind in the outer vestibule of the channel and block ion conduction by physically occluding the pore, without affecting the kinetics of channel gating (MacKinnon and Miller, 1988, 1989a; Miller, 1988; Giangiacomo et al., 1992). Binding of the peptides occurs through a reversible, bimolecular reaction which is governed by electrostatic interactions between negatively charged residues in the channel and positively charged residues in the peptide. Consequently, the association rate constant for binding of the peptide to the channel increases as the ionic strength of the external media is diminished. Evidence supporting the idea that such peptides function as pore blockers comes from two types of experiments. The first type of evidence comes from the interaction between peptide toxins and TEA, which blocks some potassium channels by binding to a site in the pore close to the external entrance (Villarroel et al., 1988). TEA inhibits the rate of association of ChTX and IbTX with some potassium channels in proportion with channel occupancy by TEA, but has no effect on toxin dissociation rate (Miller, 1988; Giangiacomo et al., 1992). This finding suggests that the binding sites for these ligands overlap. In the second type, the rate of toxin dissociation from the channel was determined in the presence of different concentrations of monovalent cations present at the inner face of the channel. The off rates of the toxins increased as the concentrations of permeant monovalent cations on the internal side was increased (MacKinnon and Miller, 1988; Giangiacomo et al., 1992; Goldstein and Miller, 1993). These data suggest that permeant ions occupy a site along the conduction pathway that is in close physical proximity to the receptor site for the peptide. Occupancy of that site by permeant ions destabilizes inhibitor binding, presumably by electrostatic repulsion with a positive charge on the toxin molecules. The positive charge on ChTX that mediates this effect has been localized to the side chain of Lys_{27} (Park and Miller, 1992a,b; Goldstein and Miller, 1993).

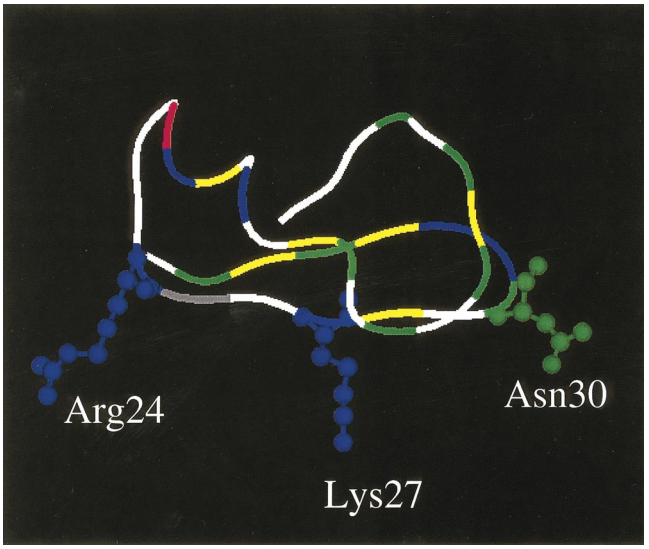

Fig. 2. Backbone structure of $AgTX_2$. The solution structure used for the display was from (Krezel et al., 1995). The side chains of Arg_{24}, Lys_{27}, and Asn_{30} are shown. These residues when individually mutated to Ala cause a large destabilization on the peptide's interaction with the *Shaker* K^+ channel (Ranganathan et al., 1996). Colors represent residues with the following properties: white, hydrophobic; green, polar; red, negatively charged; blue, positively charged; gray, aromatic; yellow, cysteine.

Understanding the pore-blocking mechanism of ChTX and related peptides provided a powerful tool to identify channel residues that form the pore and the external surface surrounding the pore. The first indications that the *Shaker* potassium channel pore region may be formed by residues between S_5 and S_6 came from mutagenesis experiments that identified residues affecting ChTX block (MacKinnon and Miller, 1989b; MacKinnon et al., 1990). Amino acids directly involved in toxin binding to the *Shaker* potassium channel were then identified using site-directed mutagenesis and mutant cycle analysis (Goldstein et al., 1994; Hidalgo and MacKinnon, 1995; Gross and MacKinnon, 1996; Ranganathan et al., 1996) and are localized between $K_v1.X$ transmembrane domains S_5 and S_6. Detailed biophysical and pharmacological analysis of specific mutations introduced into this sequence stretch lead to the conclusion that the pore-forming region of the channel is composed of residues located in the S_5–S_6 linker and demonstrated that a G–Y–G signature sequence present in this region is crucial for conferring K^+ selectivity to the channel (Heginbotham et al., 1994). As described above, external ChTX and TEA are competitive inhibitors of the high-conductance Ca^{2+}-activated K^+ (maxi-K) channel (Miller, 1988). When the residue of the *Shaker* K^+ channel that is involved in external TEA binding was identified, it was also found to be located in the S_5–S_6 linker (Heginbotham and MacKinnon, 1992). Thus, the mutagenesis experiments happily provided a structural rationale for the biophysical finding that the binding sites for ChTX and TEA overlap.

The S_5–S_6 linker is the only region of the potassium channel that is directly responsible for the peptide's high-affinity interaction with potassium channels. When the S_5–S_6 linker was transferred from the highly toxin-sensitive $K_v1.3$ channel to the toxin-insensitive $K_v2.1$ potassium channel, the resulting chimeric channel acquired sensitivity to $AgTX_2$ (Gross et al., 1994). The finding that $K_v2.1$ is completely insensitive to all known scorpion toxin peptides may not to be due to a differently shaped ion channel entryway, but rather could be a consequence of the presence of two lysine residues placed at just the right locations to prevent binding of candidate peptide blockers. These observations, which have been extended and confirmed with $K_v3.2$ channels (Hanner et al., 1999), were the first to suggest that all voltage-dependent potassium channels have a similarly shaped ion channel entryway, and that toxin sensitivity can be conferred on all toxin-insensitive, voltage-dependent potassium channels with a conserved S_5–S_6 linker by small modifications of the channel sequence. As an example, wild-type *Shaker* is very insensitive to ChTX, but its affinity can be enhanced more that 500-fold by introducing a single mutation $Phe_{425} \rightarrow Gly$ in the external mouth of the channel (Goldstein and Miller, 1993). Apparently, the size of the residue at position 425 in the *Shaker* channel can sterically prevent peptides from reaching their binding site. A similar finding was made regarding inhibition of the $K_v1.3$ potassium channel by these peptides (Aiyar et al., 1995).

3. Characterization of peptide-channel interaction residues

Site-directed mutagenesis studies were carried out with ChTX and $AgTX_2$ to identify those residues which are

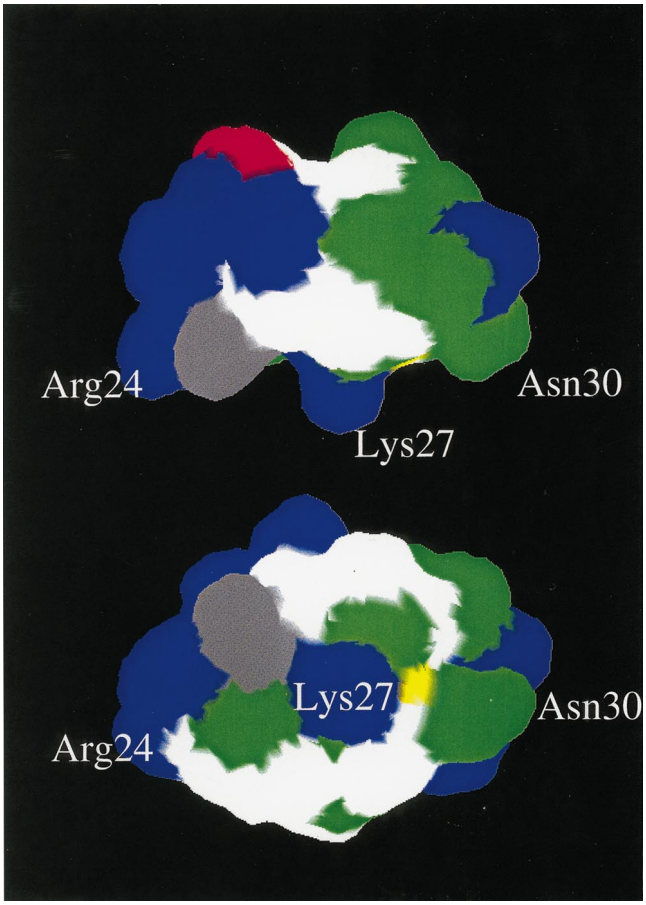

Fig. 3. Three-dimensional structure of $AgTX_2$. Two views of the interaction surface of $AgTX_2$ indicating the positions of the three crucial residues for interaction with the *Shaker* K^+ channel. Residues are colored as indicated in Fig. 2. The bottom panel shows a view of the surface from the perspective of the channel, and the top panel is rotated 90° around the *y*-axis.

important for interaction with potassium channels. These studies were enabled by the ability to biosynthetically produce large quantities of the relevant peptides, whereby recombinant peptides are made in *E. coli* as soluble fusion proteins (Park et al., 1991). Purification of the fusion protein, folding of peptide and its cleavage from the fusion protein can be easily accomplished to yield 1–5 mg of purified peptide per liter of *E. coli* culture. Several peptide residues were found to be crucial for interaction with potassium channels (Goldstein et al., 1994; Stampe et al., 1994; Ranganathan et al., 1996). Using knowledge of the three-dimensional structure of the peptide, it was determined that the active face of the inhibitor which interacts with the channel is a flat structure formed by residues from the anti-parallel β-strands (Figs. 2 and 3). One of the critical residues in ChTX that displays unique properties is Lys_{27}. Charge neutralization of this residue abolishes the effects of voltage and permeant ions on ChTX inhibition of the maxi-K channel (Park and Miller, 1992a). These results suggest that Lys_{27} of ChTX lies physically close to a potassium binding site in the ion conduction pore and, therefore, its location in the toxin-channel complex lies near the center of symmetry in the tetrameric potassium channel structure. Studies carried out with $AgTX_2$ and *Shaker* K^+ channel have also shown that Lys_{27} in the peptide comes very close to a potassium binding site in the pore (Ranganathan et al., 1996). It is expected that the shape of the ion channel receptor is complementary to that of the active face of the peptide, and must be present four times in the homotetrameric channel. These studies also led to the conclusion that the peptide interacts with residues from all four potassium channel subunits.

The toxin structures can be used as templates in mutagenesis studies to deduce the topology of the pore-forming region of the channel. In order to interpret these findings one must distinguish between effects on toxin–channel interactions that result from altering specific interactions between a pair of interacting residues, and effects that occur subsequent to more global conformational changes induced in the channel by a given mutation. These

possibilities can be distinguished by systematically mutating pairs of residues in channel and peptide and measuring the binding affinity of each combination. Thus, if amino acid X on the peptide interacts with amino acid Y on the channel, then the effect manifested by mutating residue X should depend on whether Y is mutated. This can be quantified using a thermodynamic cycle that analyzes the situation where X and Y are mutated separately, and then together (Hidalgo and MacKinnon, 1995). The coupling coefficient, Ω, is defined as:

$$\Omega = \frac{K_i(\text{wt} : \text{wt}) \times K_i(\text{mut} : \text{mut})}{K_i(\text{wt} : \text{mut}) \times K_i(\text{mut} : \text{wt})}$$

If the two mutated residues are independent of one another and, therefore, do not interact, then Ω will equal 1. If, however, the residues interact and the mutation alters their interaction, then Ω will deviate from unity. The degree of interaction, or coupling energy caused by the double mutation, is equal to $RT \ln \Omega$, where R is the gas constant and T is the absolute temperature.

When this analysis was used to study the electrostatic interactions that govern binding of $AgTX_2$ to the *Shaker* potassium channel, the pair of residues $Arg_{24}Gln–Asp_{431}Asn$ yielded a coupling energy of about 17 kJ mol^{-1}. This effect was specific, and not found with many other pairs of substitutions, supporting the idea of a local interaction between Asp_{431} and Arg_{24}. This conclusion leads to other predictions. For instance, if Lys_{27} on $AgTX_2$ is located centrally over the pore, and given the four-fold symmetry of the channel, then Arg_{31}, located on the opposite side of the peptide from Arg_{24}, should interact with Asp_{431} on the diagonally opposite channel subunit. The value of Ω for the $Arg_{31}Gln–Asp_{431}Asn$ pair corresponds to a coupling energy of 4 kJ mol^{-1}. Further analysis of these positions has indicated that Arg_{31} and Asp_{431} are within a few angstroms of each other and display through-space electrostatic coupling, while Arg_{24} and Asp_{431}, on the other hand, more likely are forming a salt bridge. The distance between residues Arg_{24} and Arg_{31} gives an indication of the spacing between Asp_{431} residues on diagonally opposed channel subunits, and imposes constraints on the structure of the pore-forming region of the channel.

Surveying all residues that are predicted to form part of the interaction surface of the peptide and channel has led to identification of other pairs of interacting residues. By placing the peptide in its binding site with Lys_{27} at the center, a toxin interaction surface can be created by rotating the peptide four-fold around Lys_{27}. The complement of this surface should exhibit the approximate shape of the external pore entryway. Mapping all channel residues displaying high coupling energy within this four-fold surface provides a low-resolution surface representation of the pore loops that form the external ion conduction pathway. The orientation of the peptide in the channel indicates that the external pore region has the shape of a shallow cone and there appears to be no large walls surrounding the vestibule (Ranganathan et al., 1996). All of these structural features are consistent with functional data regarding this region of the channel, such as the spatial location of channel residues at position 449 necessary for coordination of a tetraethylamonium ion, and the fact that only residues located on the amino-terminal side of position 448 alter selectivity. In addition to this type of analysis that defines pairs of interacting residues, side chain accessibility to sulfydryl reagents after conversion of pore loop residues to Cys reveals an interesting pattern of reactivity (Gross and MacKinnon, 1996). On the amino terminal side of the pore, all reactive residues lie on one face of a helical wheel. This implies an α-helical conformation, and suggests the presence of four helices (one from each subunit) oriented with their carboxyl terminals pointing toward a central point in the pore which could attract cations to that location because of the helical dipole moment. On the carboxyl side of the pore loop, it appears that the polypeptide chain moves radically outward in an extended conformation. In this way, functional studies with peptidyl inhibitors and potassium channels have led to prediction of a low-resolution structure of the pore region of potassium channels. It is remarkable how use of such peptides isolated from scorpion venoms led to so many predictions regarding the structure and behavior of potassium channels.

4. High-resolution structure of potassium channels

The X-ray structure of the KcsA channel at 3.2 Å reveals that the channel is a tetramer with four-fold symmetry around a central pore (Doyle et al., 1998). Each subunit consists of two α-helical transmembrane domains connected by the pore region, which consists of the turret, pore helix, and selectivity filter. In the tetrameric structure, each subunit contributes one transmembrane helix to form the central pore, while the other transmembrane domain faces the lipid environment. The inner helices are tilted with respect to the plane of the membrane by about 25° and are slightly kinked. They pack against each other as a bundle near the intracellular face of the membrane, giving the appearance of an inverted teepee. The pore helices are oriented in an amino to carboxyl direction pointed towards the center of the channel. The narrow selectivity filter is only 12 Å long and the remainder of the internal pore is wider and has a relatively inert hydrophobic lining. The K^+ selectivity filter is lined by carbonyl oxygen atoms. When an ion enters the selectivity filter, it dehydrates. To compensate for the energetic cost of lost water, the carbonyl oxygen atoms come in close contact with the ion and stabilize it. The structure of the selectivity filter prevents the carbonyl oxygen atoms from similarly compensating for dehydration of a Na^+ ion. This feature of the selectivity filter explains the K^+ selective properties of the channel. The selectivity filter contains two K^+ ions located at opposite ends, separated by 7.5 Å. The structure of the selectivity filter indicates that a single K^+ ion would be held very tightly, which

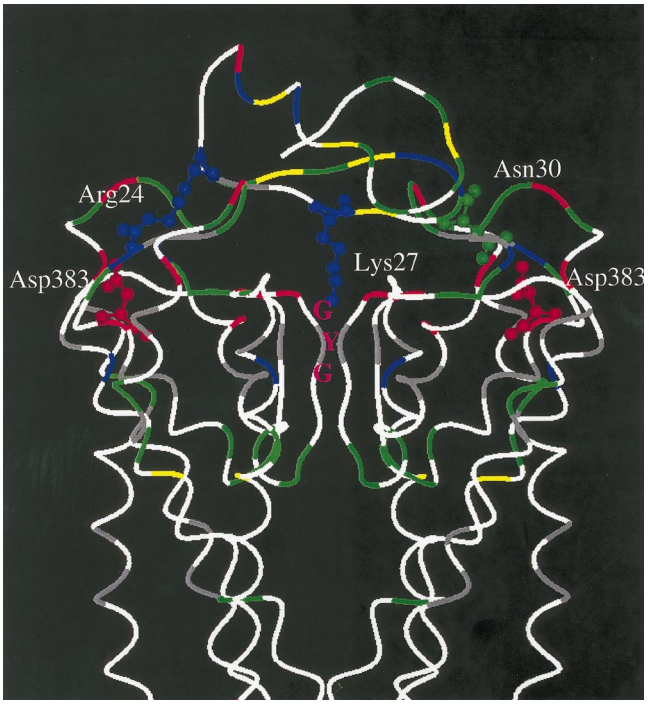

Fig. 4. Homology model of the pore region of *Shaker* K^+ channel. The KcsA structure (1BL8) was used as a template to create a model of the *Shaker* K^+ channel using the Modeler module with InsightII. The sequence alignment of KcsA and *Shaker* K^+ channel is the same as that described (Doyle et al., 1998). $AgTX_2$ has been docked in the external vestibule of *Shaker* K^+ channel. The side chains of the three critical residues in $AgTX_2$ are indicated as well as the positions of the two Asp_{431} residues in two different channel subunits that are predicted to make interactions with either Arg_{24} or Asn_{30} in $AgTX_2$. Residues are colored as indicated in Fig. 2. Notice the existence of four subunits folded to form a tetrameric channel. The G–Y–G signature sequence in the selectivity filter is colored in magenta. The cavity found below the selectivity filter is filled with water, and, together with the four pore helixes, contributes to minimizing the electrostatic barrier that a potassium ion would face when it crosses the lipid bilayer.

would limit ion flux to low levels. The presence of two K^+ ions in the structure solves the apparent paradox of high selectivity and high throughput. Mutual repulsion between the potassium ions leads to destabilization, allowing more than a million ions per second to pass through a channel. The KcsA channel possesses a 10 Å diameter cavity below the selectivity filter in the center of the channel with a hydrated cation in it. Electrostatic calculations indicate that when an ion moves through the membrane it must cross an energy barrier that is maximal at the center of the membrane. At the center of the bilayer, the polarizability of the surrounding medium is minimal and the energy of the cation is highest. Thus, by surrounding an ion with water, the cavity overcomes the electrostatic destabilization that results from the low dielectric constant of the bilayer. In addition, the four pore helices point directly at the center of the cavity and their amino to carboxyl orientation will impose a negative electrostatic potential due to a helix dipole effect. Thus, the aqueous cavity and the oriented helices help to lower the electrostatic barriers that a cation faces in crossing a lipid bilayer (Roux and MacKinnon, 1999).

The KcsA channel is not sensitive to known peptidyl inhibitors isolated from scorpion venoms. In other potassium channels, functional studies have mapped the binding sites for these peptides to regions corresponding to the extracellular entry way of the KcsA channel. Although the amino acids of the K^+ channel selectivity filters are highly conserved, the residues lining the entry way are quite variable within K^+ channels, suggesting that although the three-dimensional structure of this region may be similar, the precise amino acid composition is not identical. Because the affinity of a given peptide–channel interaction depends on the residue match on the interaction surfaces, it may be possible to confer toxin sensitivity to an insensitive channel by modifying the channel's interaction surface. Before knowledge of the X-ray structure of potassium channels, an empirical approach was used to confer $AgTX_2$ sensitivity to the voltage-gated K^+ channel, $K_v2.1$ (Gross et al., 1994). However, if the structure of the KcsA channel is relevant to that of other K^+ channels, then it should be possible to render KcsA sensitive to peptidyl inhibitors by taking advantage of the knowledge base regarding these peptides' interaction with the *Shaker* K^+ channel. Comparison of pore

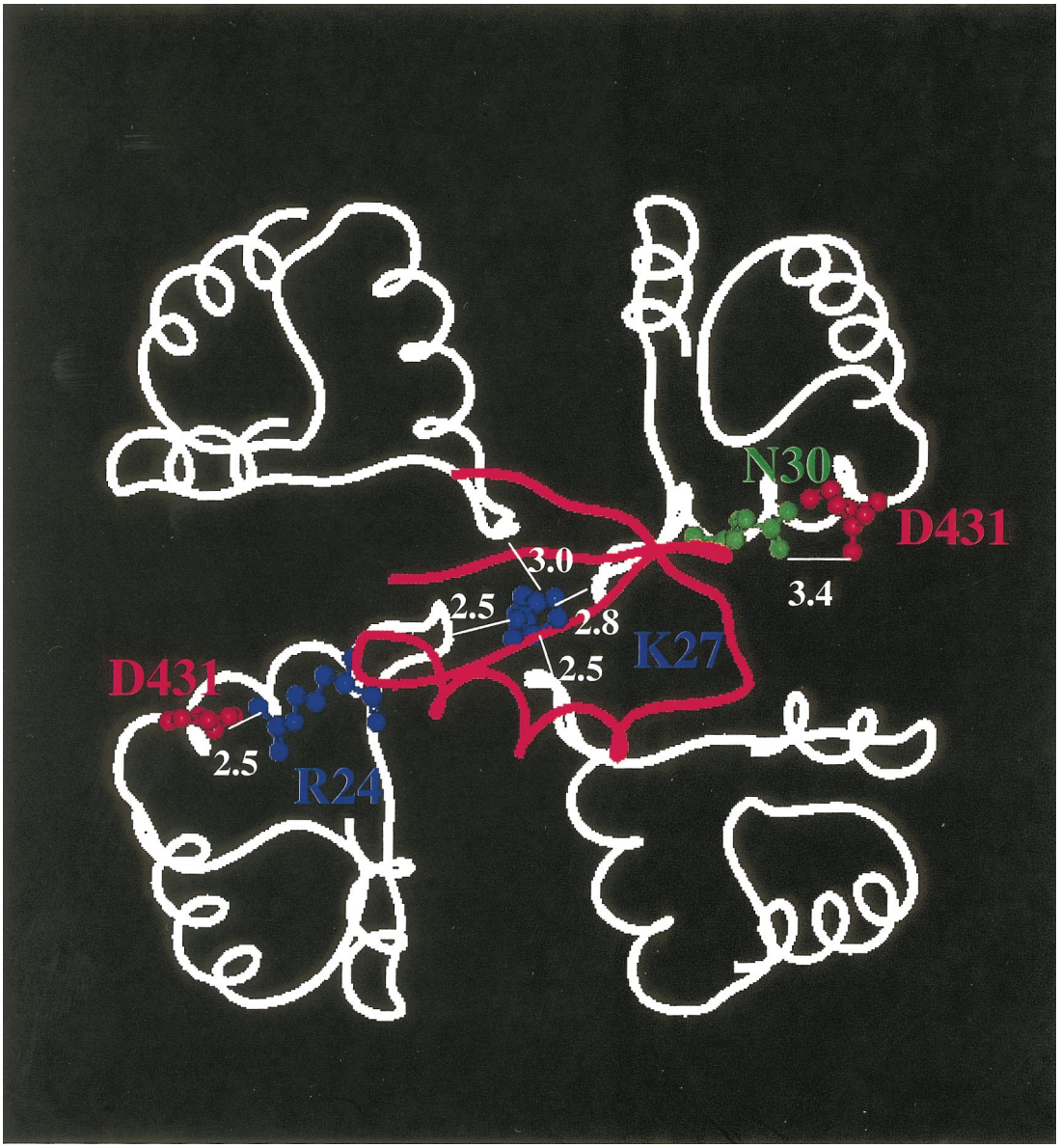

Fig. 5. Homology model of the pore region of *Shaker* K^+ channel. Top view of *Shaker* K^+ channel docked with $AgTX_2$. The side chains of Arg_{24}, Lys_{27}, and Asn_{30} in $AgTX_2$ and Asp_{431} of two different and opposing subunits in the *Shaker* K^+ channel are illustrated. The numbers refer to distances between: (a) side chains of Arg_{24} and Asp_{431} that form a salt bridge; (b) side chains of Asn_{30} and Asp_{431} that are predicted to form hydrogen bond; (c) side chains of Lys_{27} and the backbone carbonyls of Tyr_{445} that form hydrogen bond.

region sequences of KcsA and *Shaker* K^+ channels has identified a number of non-conserved residues that are critical in *Shaker* for its high affinity interaction with $AgTX_2$. Thus, when three of these mutations are introduced into the KcsA channel, and the modified KcsA K^+ channel is purified and immobilized on a solid support, application of crude *Leiurus quinquestriatus hebraeus* venom leads to retention of a number of peptides through affinity chromatography that upon elution were identified by mass spectroscopy as $AgTX_2$, ChTX, and Lq_2 (MacKinnon et al., 1998). In addition, although no binding of a radiolabelled derivative of $AgTX_2$ could be observed to wild-type KcsA K^+ channels, this radioligand binds with good affinity to the modified channel with a 1:1 stoichiometry. Mutations in the peptide that have large destabilizing effects on interaction with *Shaker* K^+ channels, also decreased the affinity of toxin for the modified KcsA channel, suggesting that $AgTX_2$ binds in the same manner to both the mutant KcsA and

Shaker K^+ channels. This suggests that the overall structure of the $AgTX_2$ receptor is very similar on both channels. Given these findings, it should be possible to dock $AgTX_2$ in the KcsA channel structure by making use of knowledge of which residues at the pore–toxin interface are energetically coupled in thermodynamic mutant cycle analyses. When the four best defined residue pairs are displayed in matched colors on the KcsA K^+ channel and $AgTX_2$ surfaces, a unique orientation becomes evident for the peptide on the channel. If the peptide is placed with Lys_{27} at the center, the colors match well in three dimensions, and the peptide seems to fit perfectly into the vestibule of the K^+ channel (MacKinnon et al., 1998). The four-fold symmetry of the channel provides four energetically identical orientations by which the peptide can bind. Alternatively, $AgTX_2$ could be docked on a *Shaker* K^+ channel structure derived from molecular modeling with the KcsA channel using the same type of constraints. In this model of the S_5–S_6 transmembrane regions of the *Shaker* K^+ channel (Fig. 4), docking of $AgTX_2$ reveals many of the features previously discussed for the peptide's interaction with this channel. Thus, the close proximity between Arg_{24} in the peptide and Asp_{431} in the channel becomes evident after placing Lys_{27} of $AgTX_2$ in the channel's center of symmetry (Fig. 5). Moreover, the existence of through-space electrostatic coupling between Arg_{31} and Asp_{431} located on the diagonally opposite subunit is also consistent with this model. Other residues in $AgTX_2$ such as Asn_{30}, when mutated to Ala cause a large decrease in the peptide's affinity for the *Shaker* K^+ channel (Ranganathan et al., 1996). However, the corresponding interacting residue in the channel has not been identified in functional studies. A prediction from the modeling studies is that Asn_{30} could hydrogen bond to Asp_{431} in a different subunit (Fig. 5). Mutagenesis studies can be used to validate this hypothesis.

5. Rationale drug design: peptidyl blockers of potassium channels

The finding that the overall architecture of various K^+ channels is similar has obvious implications. With the use of molecular modeling and constraints derived from peptide–channel interaction studies, it should be feasible to derive a picture of the interaction surface of other channels, and to design peptides that target toxin-insensitive K^+ channels. For instance, K_v2, K_v3 and K_v4 potassium channels are all insensitive to known peptide inhibitors derived from scorpion venoms (Garcia et al., 1997). The challenge is to understand, at the molecular level, why these channels are insensitive to the peptidyl inhibitors, and to specifically modify these peptides to confer binding affinity. Recent work suggests that this approach may provide specific K^+ channel blockers. When the pore regions of two K^+ channels present in human T-lymphocytes, $K_v1.3$ and the Ca^{2+}-activated K^+ channel, IK_{Ca1}, were modeled on the crystal structure of the KcsA channel, obvious differences were apparent between the channels (Rauer et al., 2000). $K_v1.3$ possesses a unique cluster of negatively charged residues in the turret region that are not present in IK_{Ca1}. Since ChTX blocks both of these channels with similar high affinities, three novel ChTX analogs were designed by introducing negatively charged residues in place of ChTX-Lys_{32}, which lies in close proximity to this cluster. These analogs block IK_{Ca1} with ~20-fold higher affinity than $K_v1.3$. Other ChTX-sensitive K^+ channels, such as $K_v1.2$ and $K_v1.6$, that also contain the cluster of negatively charged residues, are not blocked by these ChTX analogs, whereas the maxi-K channel that lacks the cluster is sensitive to inhibition by the peptides. These data are the first indication that it may be possible to use structure-based approaches to design selective inhibitors of K^+ channels. However, in these studies, only the relative affinities of peptide inhibitors for sensitive channels were modified. No attempts were made to confer toxin blockade to an insensitive channel. Although this approach may be more time consuming, it would ultimately confirm the feasibility of rationally designing inhibitors of K^+ channels. Moreover, out of all peptidyl inhibitors described so far, IbTX remains the only selective inhibitor of a K^+ channel; it blocks solely maxi-K channels. Understanding the basis of IbTX's strict specificity for maxi-K channels is another of the challenges facing ongoing structural studies of K^+ channels.

References

Aiyar, J., Withka, J.M., Rizzi, J.P., Singleton, D.H., Andrews, G.C., Lin, W., Boyd, J., Hanson, D.C., Simon, M., Dethlefs, B., Lee, C.-L., Hall, J.E., Gutman, G.A., Chandy, K.G., 1995. Topology of the pore-region of a K^+ channel revealed by the NMR-derived structures of scorpion toxins. Neuron 15, 1169.

Bontems, F., Gilquin, B., Roumestand, C., Menez, A., Toma, F., 1992. Analysis of side chain organization on a refined model of charybdotoxin; structural and functional implications. Biochemistry 31, 7756.

Bontems, F., Roumestand, C., Gilquin, B., Menez, A., Toma, F., 1991. Refined structure of charybdotoxin: common motifs in scorpion toxins and insect defensins. Science 254, 1521.

Cuello, L.G., Romero, J.G., Cortes, D.M., Perozo, E., 1998. pH-Dependent gating in the *Streptomyces lividans* K^+ channel. Biochemistry 37, 3229.

Dauplais, M., Gilquin, B., Possani, L.D., Gurrola-Briones, G., Roumestand, C., Menez, A., 1995. Determination of the three-dimensional solution structure of noxiustoxin: analysis of structural differences with related short-chain scorpion toxins. Biochemistry 34, 16563.

Doyle, D.A., Cabral, J.M., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T., MacKinnon, R., 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280, 69.

Fernandez, I., Romi, R., Szendeffy, S., Martin-Eauclaire, M.F., Rochat, H., Rietschoten, J.B., Pons, M., Giralt, E., 1994.

Kaliotoxin (1-37) shows structural differences with related potassium channel blockers. Biochemistry 33, 14256.

Galvez, A., Gimenez-Gallego, G., Reuben, J.P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, G.J., Garcia, M.L., 1990. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. J. Biol. Chem. 265, 11083.

Garcia, M.L., Garcia-Calvo, M., Hidalgo, P., Lee, A., MacKinnon, R., 1994. Purification and characterization of three inhibitors of voltage-dependent K^+ channels from *Leiurus quinquestriatus* var. *hebraeus* venom. Biochemistry 33, 6834.

Garcia, M.L., Hanner, M., Knaus, H.-G., Koch, R., Schmalhofer, W., Slaughter, R.S., Kaczorowski, G.J., 1997. Pharmacology of potassium channels. Adv. Pharmacol. 39, 425.

Garcia, M.L., Kaczorowski, G.J., 1998. Pharmacology of high-conductance calcium-activated potassium channels. Curr. Res. Ion Channel Modulators 3, 213.

Garcia, M.L., Knaus, H.-G., Munujos, P., Slaughter, R.S., Kaczorowski, G.J., 1995. Charybdotoxin and its effects on potassium channels. Am. J. Physiol. 269, C1.

Garcia-Calvo, M., Knaus, H.-G., McManus, O.B., Giangiacomo, K.M., Kaczorowski, G.J., Garcia, M.L., 1994. Purification and reconstitution of the high-conductance calcium-activated potassium channel from tracheal smooth muscle. J. Biol. Chem. 269, 676.

Garcia-Calvo, M., Leonard, R.J., Novick, J., Stevens, S.P., Schmalhofer, W., Kaczorowski, G.J., Garcia, M.L., 1993. Purification, characterization, and biosynthesis of margatoxin, a component of *Centruroides margaritatus* venom that selectively inhibits voltage-dependent potassium channels. J. Biol. Chem. 268, 18866.

Giangiacomo, K.M., Garcia, M.L., McManus, O.B., 1992. Mechanism of iberiotoxin block of the large-conductance calcium-activated potassium channel from bovine aortic smooth muscle. Biochemistry 31, 6719.

Goldstein, S.A.N., Miller, C., 1993. Mechanism of charybdotoxin block of a voltage-gated K^+ channel. Biophys. J. 65, 1613.

Goldstein, S.A.N., Pheasant, D.J., Miller, C., 1994. The charybdotoxin receptor of a *Shaker* K^+ channel: peptide and channel residues mediating molecular recognition. Neuron 12, 1377.

Gross, A., Abramson, T., MacKinnon, R., 1994. Transfer of the scorpion toxin receptor to an insensitive potassium channel. Neuron 13, 961.

Gross, A., MacKinnon, R., 1996. Agitoxin footprinting the *Shaker* potassium channel pore. Neuron 16, 399.

Hanner, M., Schmalhofer, W.A., Green, B., Bordallo, C., Liu, J., Slaughter, R.S., Kaczorowski, G.J., Garcia, M.L., 1999. Binding of correolide to K_v1 family potassium channels. Mapping the domains of high affinity interaction. J. Biol. Chem. 274, 25237.

Heginbotham, L., LeLasurier, M., Kolmakova-Partensky, L., Miller, C., 1999. Single *Streptomyces lividans* K^+ channels. Functional asymmetries and sidedness of proton activation. J. Gen. Physiol. 114, 551.

Heginbotham, L., Lu, Z., Abramson, T., MacKinnon, R., 1994. Mutations in the K^+ channel signature sequence. Biophys. J. 66, 1061.

Heginbotham, L., MacKinnon, R., 1992. The aromatic binding site for tetraethylammonium ion on potassium channels. Neuron 8, 483.

Hidalgo, P., MacKinnon, R., 1995. Revealing the architecture of a K^+ channel pore through mutant cycles with a peptide inhibitor. Science 268, 307.

Johnson, B.A., Stevens, S.P., Williamson, J.M., 1994. Determination of the three-dimensional structure of margatoxin by ^1H, ^{13}C, ^{15}N triple-resonance nuclear magnetic resonance spectroscopy. Biochemistry 33, 15061.

Johnson, B.A., Sugg, E.E., 1992. Determination of the three-dimensional structure of iberiotoxin in solution by ^1H nuclear magnetic resonance spectroscopy. Biochemistry 31, 8151.

Krezel, A.M., Kasibhatla, C., Hidalgo, P., MacKinnon, R., Wagner, G., 1995. Solution structure of the potassium channel inhibitor agitoxin 2: caliper for probing channel geometry. Protein Sci. 4, 1478.

MacKinnon, R., 1991. Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature 350, 232.

MacKinnon, R., Cohen, S.L., Kuo, A., Lee, A., Chait, B.T., 1998. Structural conservation in prokaryotic and eukaryotic potassium channels. Science 280, 106.

MacKinnon, R., Miller, C., 1988. Mechanism of charybdotoxin block of the high-conductance, Ca^{2+}-activated K^+ channel. J. Gen. Physiol. 91, 335.

MacKinnon, R., Miller, C., 1989. Functional modification of a Ca^{2+}-activated K^+ channel by trimethyloxonium. Biochemistry 28, 8087.

MacKinnon, R., Miller, C., 1989. Mutant potassium channels with altered binding of charybdotoxin, a pore-blocking peptide inhibitor. Science 245, 1382.

MacKinnon, R., Heginbotham, L., Abramson, T., 1990. Mapping the receptor site for charybdotoxin, a pore-blocking potassium channel inhibitor. Neuron 5, 767.

Miller, C., 1988. Competition for block of a Ca^{2+}-activated K^+ channel by charybdotoxin and tetraethylamonium. Neuron 1, 1003.

Miller, C., Moczydlowski, E., Latorre, R., Phillips, M., 1985. Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. Nature 313, 316.

Park, C.S., Hausdorff, S.F., Miller, C., 1991. Design, synthesis, and functional expression of a gene for charybdotoxin, a peptide blocker of K^+ channels. Proc. Natl. Acad. Sci. USA 88, 2046.

Park, C.-S., Miller, C., 1992. Interaction of charybdotoxin with permeant ions inside the pore of a K^+ channel. Neuron 9, 307.

Park, C.-S., Miller, C., 1992. Mapping function to structure in a channel-blocking peptide: electrostatic mutants of charybdotoxin. Biochemistry 31, 7749.

Possani, L.D., Martin, B.M., Svendsen, I.B., 1982. The primary structure of Noxiustoxin: a K^+ channel blocking peptide, purified from the venom of the scorpion *Centruroides noxius* Hoffmann. Carlsberg Res. Commun. 47, 285.

Ranganathan, R., Lewis, J.H., MacKinnon, R., 1996. Spatial localization of the K^+ channel selectivity filter by mutant cycle-based structure analysis. Neuron 16, 131.

Rauer, H., Lanigan, M.D., Pennington, M.W., Aiyar, J., Ghanshani, S., Cahalan, M.D., Norton, R.S., Chandy, K.G., 2000. Structure-guided transformation of charybdotoxin yields an analog that selectively targets Ca^{2+}-activated over voltage-gated K^+ channels. J. Biol. Chem. 275, 1201.

Romi, R., Crest, M., Gola, M., Sampieri, F., Jacquet, G., Zerrouk, H., Mansuelle, P., Sorokine, O., Dorsselaer, A.V., Rochat, H., Martin-Eauclaire, M.-F., Rietschoten, J.V., 1993. Synthesis and characterization of kaliotoxin. J. Biol. Chem. 268, 26302.

Roux, B., MacKinnon, R., 1999. The cavity and pore helices in the

KcsA K^+ channel: electrostatic stabilization of monovalent cations. Science 285, 100.

Stampe, P., Kolmakova-Partensky, L., Miller, C., 1994. Intimations of K^+ channel structure from a complete functional map of the molecular surface of charybdotoxin. Biochemistry 33, 443.

Tytgat, J., Chandy, K.G., Garcia, M.L., Gutman, G.A., Martin-Eauclaire, M.-F., Walt, J.J.v.d, Possani, L.D., 1999. A unified nomenclature for short-chain peptides isolated from scorpion venoms: α-KTx molecular subfamilies. TiPS 20, 444.

Villarroel, A., Alvarez, O., Oberhauser, A., Latorre, R., 1988. Probing a Ca^{2+}-activated K^+ channel with quaternary ammonium ions. Pfluge. Arch. 413, 118.