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Topical Review

Structure-Function Studies on the Pore of Potassium Channels

O. Pongs
Zentrum für Molekulare Neurobiologie, Institut für Neurale Signalverarbeitung, 20246 Hamburg, Germany

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Introduction

Potassium (K) channels occur in most, if not all, membranes of both excitable and inexcitable cells (Rudy, 1988). The activity of K-channels controls the electrical potential across the cell membrane and thereby may regulate cellular processes such as cell excitability, secretion, and signal transduction. The great variety of K-channel function apparently requires that K-channels be highly diverse in their electrophysiological and gating properties in order to meet the specific physiological needs in a given cell.

The successful cloning of a number of voltage- and ligand-gated K-channels (Catterall, 1992; Pongs, 1992*a*,*b*) has shed considerable light into their structure and function. Combinations of molecular biological and electrophysiological studies have paved the ground to develop general concepts for understanding such basic K-channel properties as gating, inactivation, and ion selectivity. Also, the cloning of many different K-channel genes has revealed that K-channels belong to a large superfamily of ionic channels, which does not only include voltage- and ligand-gated K-channels, but also Na and Ca channels (Jan & Jan, 1990). The unexpected kinship among all these different ionic channels may have general implications for the structure of ionic channels. This review is about the pore region of K-channels, with little emphasis on the specific properties of the individuals. Although we are far from understanding the molecular properties of K-channels in detail, there has been exciting progress in the characterization of the pore region and in the understanding of ion transport through the pore. Recently, reviews have appeared on several aspects of the molecular biology and electrophysiology of K-channels. For further information the reader is referred to those (Catterall, 1992; Jan & Jan, 1992; Miller, 1992; Pongs 1992*a*,*b*; Salkoff et al., 1992).

Key words: Potassium channels — *Xenopus* oocytes — Ion selectivity — Sodium channels — Calcium channels — Membrane protein structure

Background

Based on the amino acid sequences of various Na-, Ca- and K-channel proteins, topological models have been proposed to explain how the channel proteins fold to form the ion channel pore (Guy & Conti, 1990; Durrell & Guy, 1992). The Na- and Ca-channel proteins fold consists of four domains which arrange themselves in a symmetrical manner to form the lining of the pore. The amino- and carboxy-terminus of each domain are facing the cytoplasmic side of the membrane. The core of each domain is inserted into the membrane such that it probably transverses the lipid bilayer six times. Five transmembrane segments (S1-S3, S5, S6) have a hydrophobic character according to Kyte-Doolittle (1982) criteria. The fourth transmembrane segment S4 appears to be a hallmark of voltage-gated ion channels. It carries a number of regularly spaced, positively charged amino acid side chains (lysine or arginine) exhibiting the general sequence pattern (Arg/Lys-X-X)*n*, where X may be almost any hydrophobic or hydrophilic amino acid and *n* may vary between 4 and 7 (Pongs, 1992*a*). The S4 segment is thought to be involved in the gating of the channel by acting as a sensor for changes in the electrical field across the membrane (Hille, 1992). The sequence joining transmembrane segments S5 and S6 in Na-, Ca- and K-channels greatly varies in length and in sequence. However, it invariably contains a hydrophobic stretch of 19 amino acids that is conserved among each type of ion channel (Guy & Conti, 1990). This

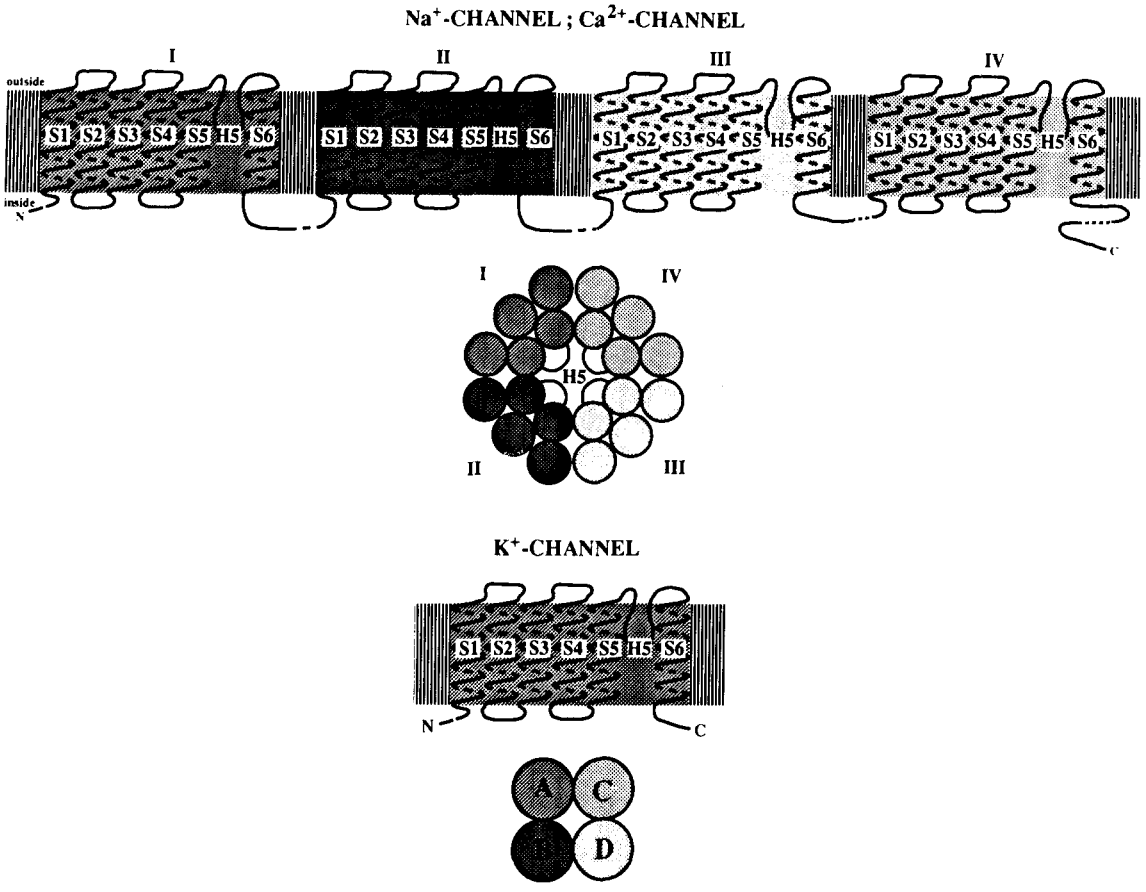

Fig. 1. Proposed membrane spanning orientation of proteins forming voltage-gated Na^+ and Ca^{2+} channels (top) or voltage-gated K^+ channels (bottom). Lipid bilayer is shaded in gray. Domains I, II, III, IV of Na^+/Ca^{2+} channels have a similar topology. Putative membrane spanning segments S1-S6 are indicated, assuming an α-helical secondary structure. The pore-forming H5 sequence enters and exits the lipid bilayer from the extracellular side. *N*: amino-terminus; *C*: carboxy-terminus. Probably, domains I, II, III, IV fold to form a central pore. A top view of hypothetical fold is schematically indicated. Circles correspond to segments S1 to S6 of each domain. The H5 sequence is at the center. In the case of K^+ channels, a tetrameric assembly of subunits is assumed. Each circle indicates one subunit. The pore is at the center. The subunits are labeled *A, B, C, D* to indicate that K^+ channels may be heteromultimeric assemblies of different subunits.

stretch of amino acids has been named the P-region (P for pore), H5 or SS1/SS2 (Durell & Guy, 1992). The H5 segment is tucked into the membrane entering and exiting the membrane from the extracellular side. The present structural models predict that each Na- and Ca-channel domain contributes one H5 segment to form the lining of the pore. Since the K-channel protein fold is similar to one Na-/Ca-channel domain, four K-channel subunits may assemble into a tetramer (MacKinnon, 1991), which provides the pore lining. Results from various recent studies favor a folding model of channel proteins which is common to voltage-gated Na-, Ca- and K- channels (Fig. 1). A deduction commonly made from hydropathy plots is that the transmembrane portion of each subunit/domain is composed of six α-helices surrounding the H5 segment which has been modeled as a β-sheet (Durell & Guy, 1992). However, no direct experimental evidence is available in support of this arrangement. Recent electron microscopy results on the acetylcholine receptor, a neurotransmitter-gated ionic channel, suggest a different folding arrangement (Unwin, 1993). The acetylcholine receptor may be made up from five α-helices, one of each subunit (Unwin 1989, 1993). The α-helical pore is surrounded by a β-sheet transmembrane portion formed by three transmembrane segments of each subunit. Furthermore, X-ray diffraction studies of porins have shown that they form a pore across a bilayer membrane by folding its polypeptide chain in the form of a 16-strand β-barrel (Weiss et al., 1991; Cowan et al., 1992). Subunits with an all α-helical transmembrane structure require matching side chain to side chain contacts for assembly within the lipid bilayer. β-sheet-rimmed subunits may not need this because they could form a stable structure

within the lipid bilayer by hydrogen bonding between the polar backbone carbonyl and amide groups. The assembly of subunits would not depend on exact matching of residues across their interfaces. β-sheet structures may well be the structural basis for the observation that domain swapping experiments can be carried out easily among members of the K-channel superfamily. Also, it could account for results which suggest that the specificity of subunit assembly among various K-channel subunits depends on cytoplasmic amino-terminal sequences that are not part of the transmembrane portion of the channel (Li, Jan & Jan, 1992). A corollary of this hypothesis is (i) that subunit recognition is determined by side chain to side chain interactions between channel subunit sequences which lie outside of the membrane on the cytoplasmic side; and (ii) that subunit stoichiometry is determined by β-sheet associations within the lipid bilayer, until most or all exposed hydrogen bond-forming groups are saturated and a β-sheet barrel is formed. This type of assembly mechanism potentially could give ion channel assembly an enormous flexibility. Seemingly minor changes in sequence could result in the formation of β-sheet barrels having different diameters. Larger β-barrels would be assembled by a higher number of subunits creating pores of larger size. Smaller β-barrels would be assembled by a smaller number of subunits creating pores of smaller size.

On the basis of sequence comparisons, a structural and functional connection between the pores of Na-, Ca- and K- channels appears to exist. Before discussing the site-directed mutagenesis and single channel current analyses in detail, the most salient features of the pore of Na-, Ca- and K-channels are briefly summarized. This summary inadvertently contains some oversimplifications and generalizations for reasons of clarity. Ionic permeability ratios for many ions have been determined with Na-, Ca- and K-channels (Hille, 1992). The data suggest that the pore of these channels is not simply a molecular sieve, which allows ions of equal or smaller pore size to pass. Ions must bind to certain sites in the pore as part of the permeation process. These sites can bind only one ion at a time. One or more ion binding sites contribute to the ion selectivity of the pore. They form the selectivity filter of the channel. Furthermore, the binding site(s) contribute to the conductance properties of the pore, but are not the sole determinants. In principal, one can distinguish between one-ion pores and multi-ion pores. Both types of pore may have several internal ion binding sites, but the former can only bind one ion at a time, the latter can bind more than one ion at a time. The pores of voltage-gated Na-, Ca-, and K-channels seem to hold more than one ion at a time. For example, delayed rectifier type K-channels may hold at least three K^+ ions simultaneously in single file (Hille, 1992). This necessitates a long multi-ion pore to accommodate three K^+ ions. If one assumes that the K^+ ions permeate after having shed all but one water molecule, each ion occupies probably a radius of 4.05 Å. Taking into account electric repulsion between the K^+ ions traveling through the pore, this simple consideration tells us that multi-ion pores such as K-channel pores should be very long. Moreover, theoretical considerations suggest that each end of the channel has a high energy barrier for the ions to enter and, respectively, to exit the pore, whereas the energy barriers within the channel are low allowing rapid back and forth movements of the ion (Hille, 1992). When three K^+ ions were simultaneously bound within the pore of a K-channel, three separate binding sites must be inferred. Each binding site might contribute to the conductance properties of the K-channel, but not necessarily to its permeability or selectivity. The binding sites postulated from biophysical experiments should have structural correlates in known K-channel protein sequences.

Structure of the K-channel pore

Domain-swapping experiments and in vitro mutagenesis studies have clearly demonstrated that the H5 segment is, almost beyond doubt, the major pore forming structure of voltage-gated K-channels (Hartmann et al., 1991; Yool & Schwarz, 1991; Kirsch et al., 1992) and probably also of voltage-gated Na- and Ca-channels (Heinemann et al., 1992). However, interpretations made from mutagenesis experiments are always subject to criticisms unless they are corroborated by other results. A major concern is how much an altered amino acid chain has created a nonlocalized structural perturbation that leads to altered channel properties. Very probably, the substitution of amino acid residues which are important in forming the pore structure and/or in determining pore properties, always creates a more or less significant conformational alteration of the pore to accommodate the altered amino acid side chain.

In the lucky case, mutations of the H5 sequence cause a change in a singular and distinct pore property, e.g., conductance, selectivity, pharmacology, inactivation, K^+ dependence or Mg^{2+} block; in the worst case, the mutations lead to alterations of many channel properties or even to inactive channels. In Fig. 2, mutations that have generated nonfunctional K-channels are indicated along the sequence of the H5 segment of the *Shaker* channel. These results show that the activity of K-channels is very sensitive to perturbations in the H5 sequence. However, these mutations cannot indicate amino acid side chains

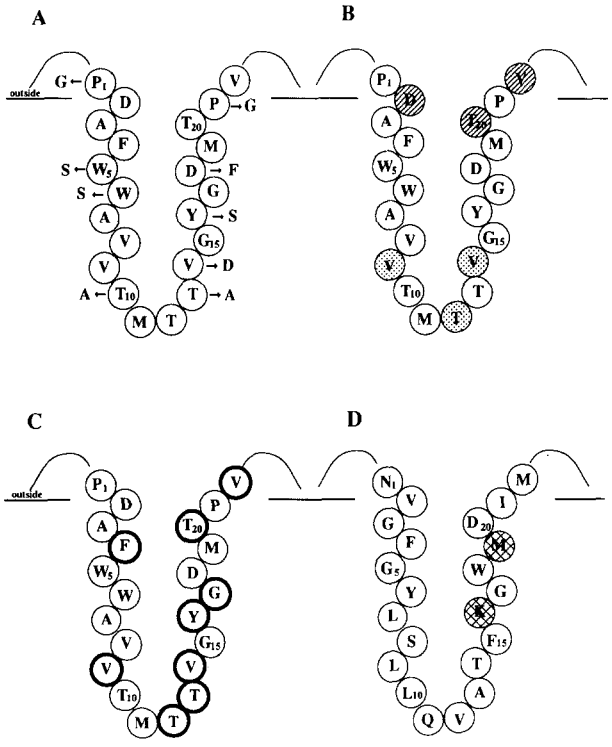

Fig. 2. Diagram in single-letter code of amino acid sequence in the pore-forming H5 region of voltage-gated *Shaker* K^+ channels (*A–C*) or of voltage-gated rat brain Na^+ channel II (*D*), inserted into the membrane from the extracellular side. (*A*) Arrows indicate amino acid replacements which lead to inactive channels. Data are from Yool and Schwarz (1991) and Lichtinghagen et al. (1990). (*B*) Amino acids affecting binding external tetraethylammonium are shaded; amino acids, affecting binding internal tetraethylammonium are stippled. Data are from McKinnon and Yellen (1990), Yellen et al. (1991), Kirsch et al. (1992). (*C*) Amino acids affecting single channel conductance, potassium dependence and/or selectivity are outlined by thick circles. Data are from Yool and Schwarz (1991), McKinnon and Yellen (1990) and Kirsch et al. (1992). (*D*) Amino acids involved in binding tetrodotoxin or saxitoxin are hatched. Data are from Terlau et al. (1991). Converting K14 to E confers Ca^{2+} channel properties upon the Na^+channel (Heinemann et al., 1992). Note that the drawing of the pore-forming region does not imply which amino acid side chains contribute directly to lining the wall of the pore. The drawing is not meant to suggest a three-dimensional structure. It solely indicates that the H5 sequence is entering and exiting the membrane from the extracellular side. The exact structure of this hairpin, and the angle at which it dips into the lipid bilayer, are not known. A hypothetic atomic scale structure of the hairpin has been proposed (Durell & Guy, 1992). In this model, the first five residues are modeled as a short α-helix, residues 6 to 12 form a β-strand, residues 13 to 15 the turn of the hairpin, and residues 16 to 21 the second β-strand.

which a potassium ion may encounter when it passes through the pore. Fortunately, a few mutations have been found in voltage-gated K- and Na-channels which distinctly alter channel properties. In this context, it should be realized that point mutations in K-channel proteins lead to the formation of tetramers having four mutations, one in each subunit; point mutations in Na-channels, on the other hand, affect only one of the four putative pore lining domains. The hypothesis that H5 is tucked into the membrane is based mainly on two types of experiments: (i) block by tetraethylammonium from the inside as well as from the outside; (ii) alteration of ionic selectivity. As indicated in Fig. 2*B*, one particular amino acid chain, close to the carboxyterminal end of the H5 segment, is a prominent determinant of the binding site for external tetraethylammonium (MacKinnon & Yellen, 1990). This site lies just inside the electrical field. Based on a systematic mutagenesis study, it has been proposed that the amino acid chains, which confer to the channel a high tetraethylammonium affinity, directly interact with the channel blocker (Heginbotham & MacKinnon, 1992). Mutation of another amino acid chain almost in the middle of the H5 segment (T441S in *Shaker* B) leads to a tenfold decrease of the K-channel's affinity for internal tetraethylammonium. The same mutation also alters the ionic selectivity of the *Shaker* K-channel which, in comparison to wild type channels, became more permeable for Rb^+ and NH_4 ions (Yool & Schwarz, 1991).

The results of these mutagenesis studies have much influenced our view of the channel pore. Also, the studies have given an important support to an atomic scale model of K-channels, obtained by molecular modeling considerations (Durrell & Guy, 1992). The folding model predicts that the H5 sequences adopt four β-hairpin structures, one from each K-channel subunit. The first eight and the last three amino acids are positioned near the extracellular entrance of the pore, whereas the turn of the β-hairpins faces the intracellular entrance of the pore. According to Guy and coworkers (1990, 1992), the turn between the first and second strand of the hairpin is made by the residues TVG in Fig. 2*B*. Note that I have displaced the turn by two residues, making the second hairpin two amino acid residues longer. This modification is based on results of in vitro mutagenesis experiments that change the selectivity of the channel (Kirsch et al., 1992). For further details the reader is referred to the legend of Fig. 2. The model of the K-channel pore predicts that the external tetraethylammonium binding site is close to the proposed external entrance. This is consistent with the effects of mutations as indicated in Fig. 2*B*. The internal binding site might be close to the turn of the hairpins. The narrowest part of the pore, which probably is identical with the selectivity filter, should be located between the internal and external tetraethylammonium binding sites. Accordingly, major K^+ binding sites in the pore should be formed

by residues in the β-hairpin structure. Then the following amino acid side chains may line the pore (numbering as in Fig. 2): F2, W4, V6, T10, Y16, D18, T20. Several recent experiments (Yool & Schwarz, 1991; Kirsch et al., 1992; Heginbotham, Abramson & MacKinnon, 1992) lend support to this hypothesis. Chimeric constructs between two H5 segments of vertebrate voltage-gated K channels (Hartmann et al., 1991) combined with in vitro mutagenesis studies (Kirsch et al., 1992) have implicated three amino acid residues in internal tetraethylammonium binding and in ionic selectivity (Fig. 2*B* and *C*). One simple interpretation is that residues V9 and V14 lie opposite to each other and that T12 is at the turn of the hairpin; on the other hand, mutating residue T13 to S between T12 and V14, has dramatic effects on K-channel selectivity and voltage dependence of activation/inactivation (Yool & Schwarz, 1991). This may indicate a relatively large nonlocalized structural perturbation of the pore, induced by the T13 to S mutation. However, changing V9 to I also affects the kinetics of activation/inactivation (Kirsch et al., 1992). Furthermore, mutations at F4 (Yool & Schwarz, 1991) and deletion of Y16 and G17 (Heginbotham et al., 1992) change the selectivity of the channel, and mutations of T20 and V22, the conductance (MacKinnon & Yellen, 1990; Kirsch et al., 1992). Combining the results, summarized in Fig. 2 *A–C*, indicates that the sequence of the H5 segment is an important determinant of K-channel pore properties. However, it is presently not clear if any one of the mutations listed in Fig. 2 alters the lining of the pore without perturbing the conformation of the pore.

Voltage-gated Na- and Ca-channels may have similarly structured pores in comparison to K-channels (Guy & Conti, 1990). This notion has found strong support by a set of site-directed mutations which were introduced into the H5 β-hairpin sequences of a rat brain voltage-gated Na-channel (Terlau et al., 1991; Heinemann et al., 1992) (Fig. 2*D*). These mutations have altered the sensitivity to the blockers tetrodotoxin and saxitoxin and affect single channel conductances. The substitutions (K1422E or A1714E in rat brain sodium channel II) in the β-hairpins of domains III and IV conferred calcium channel characteristics on the sodium channel. The results indicated that K1422E and A1714E may be close to or be part of the selectivity filter of the channel and may be in a similar position within the pore forming hairpin as are the corresponding K-channel residues. Whether charge interactions between amino acid side chains and passing ion are in general precise enough to explain selectivity is, however, still a matter of conjecture.

The open channel pore is presumably filled with water. This water has to have a high degree of order (entropy) within the pore. Consequently, an ion that enters the water-filled pore has to displace water molecules. Any change within the pore which influences the interaction of water with the wall of the pore and/or the displacement reaction between pore-entering ion and water, may change the properties of the pore. Also, a water-filled pore around its narrowest part may be an instable structure (note that single channels are usually closed in the resting state). Hydrophobic residues within a pore are expected to interact preferably with each other rather than with water in order to minimize the high entropy required for ordering surrounding water molecules. These considerations are fairly general but they predict that any conformational alteration within the pore, which affects the filling of the pore with water, must also affect the passage of ions through the pore. Therefore, properties of the channel pore could be affected in a comparable manner by seemingly different substitutions at the same amino acid position. There are a few examples where this has been investigated. Most notably, a systematic mutation of position 20 in the P-region (Fig. 2*A–C*) has indicated that several amino acid substitutions have similar effects on rapid external closure of the pore and on the K^+ dependence of the channel function (Lopez-Barneo et al., 1993). The results are difficult to explain by considering particular amino acid side chain parameters like size, hydrophibicity, hydrophilicity, steric hindrance, dipol moment and so on. An alternative interpretation could be that certain amino acid residues at position 20 perturb the arrangement of water in the pore, thereby conferring rapid closure of the channel. Some K-channels like RCK4 require external K^+ for conducting K^+ ions from the inside to the outside. It has been demonstrated that external K^+ is not required for gating of these channels. Furthermore, the K^+ dependence of RCK4 has been shown to depend on the presence of lysine at position 20 (Pardo et al., 1992). Thus, external K^+ is required for RCK4 channels to transit from the activated closed state to the open state, i.e., to get opened or, respectively, to stay open. Most probably, external K^+ is required for RCK4 channels to stay open and/or to stabilize the open state of RCK4 channels. This observation may imply that external K^+ binds within the pore of RCK4 channels. According to this hypothesis, lysine at position 20 in the H5 region would perturb the filling of the pore with water such that in the absence of external K^+ the pore cannot be stabilized in the open state. In this context, it is interesting to note that a substitution at position 9 from V to I also makes the channel opening dependent on external K^+ (Kirsch et al., 1992). This residue lies presumably deep in the pore. Again,

the effect of this mutation might be that the opening channel cannot be properly stabilized in the absence of external K^+ because of a perturbation in the interaction of the pore wall with water.

The Gate

Ionic channels have a physical gate. In the resting state, the gate keeps the channel closed. Upon activation, the gate opens and ions may flow through the pore along the electrochemical gradient (Hille, 1992). Closing the channel puts the gate into place again. These processes involve considerable conformational changes of the channel protein which are poorly understood. In vitro mutagenesis studies have been carried out with *Shaker* channels which may indicate the domain of the K-channel protein that may form the gate of the channel (Isacoff, Jan & Jan, 1991). It has been shown that rapid inactivation of the *Shaker* K-channel from the cytoplasmic side is brought about by binding of an amino-terminal "ball" peptide sequence to the cytoplasmic entrance of the pore (Zagotta, Hoshi & Aldrich, 1990). The binding site for the "ball" peptide involves amino acid side chains located between the S4 and S5 segments (Isacoff et al., 1991). The S45 sequence has been modeled as an α-helix (Durell & Guy, 1992). Mutations along one face of the S45 α-helix (A4V, T7A, S11 A, and E14Q in Fig. 3) alter voltage dependence of activation/inactivation, recovery from inactivation and single channel conductance. The results are compatible with the idea that the S45 α-helix is part of the physical gate of the K-channel. When the voltage sensor (S4 segment) moves within the electric field upon depolarization, this in turn would move the gate and the channel could open (Guy & Durrell, 1992). Note that the common structural hypothesis is that S4 and S45 are α-helices. This means that the movement of S4 and S45 takes place along the helical screw axis. Binding of the amino terminal "ball" peptide would lock the gate in the open conformation and at the same time plug the cytoplasmic entrance of the open pore, thereby inactivating the K-channel. Upon unbinding of the "ball" peptide the then open pore could close again by putting the gate back into place (Ruppersberg et al., 1991). According to this hypothesis, mutations in the gate should lead to changes in both activation, inactivation and recovery from inactivation. In addition, these mutations reduce the conductance of the channel for outward and inward current (Isacoff et al., 1991). Again, the effects of these mutations are not well understood. One possibility may be that the mutant pore becomes more amenable to a block by e.g., Mg^{2+}, which binds within the pore of K-channels. However, presently there are no data available in support of this hypothesis.

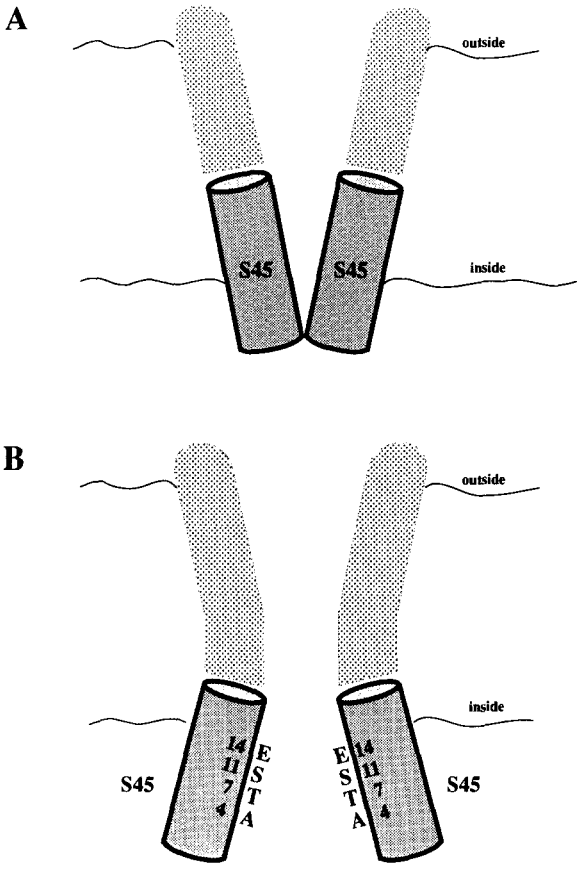

Fig. 3. Hypothetical gate of voltage-gated K-channels. Mutations in the sequence joining S4 and S5 segments of *Shaker* K-channels (Isacoff et al., 1991)—S45 alters the activation/inactivation behavior and single channel conductance for outward as well as inward currents. S45 has been modeled as an α-helix in an atomic scale model of K-channels (Durell & Guy, 1992). Residues A4, T7, S11, and E14 are located on one side of the S45 α-helix, facing the open K-channel pore. The wall of the pore has been stippled. Lipid bilayer is indicated by wavy lines. *Outside*: extracellular; *inside*: intracellular side of the membrane. Two S45 helices are shown. Possibly, four S45 helices may form the internal gate by turning into and out of the ion conduction pathway. (*A*) Hypothetical model for the closed pore in the resting state. (*B*) Hypothetical model for the open pore.

Is There a Minimal Structural Fold for K-Channels?

Recent cloning of the minK-channel (for review, *see* Philipson & Miller, 1992), of an ATP-regulated K-channel (ROMK1) (Ho et al., 1993) and an inwardly rectifying K-channel (IRK1) (Kubo et al., 1993) has indicated that K-channels do not require six hy-

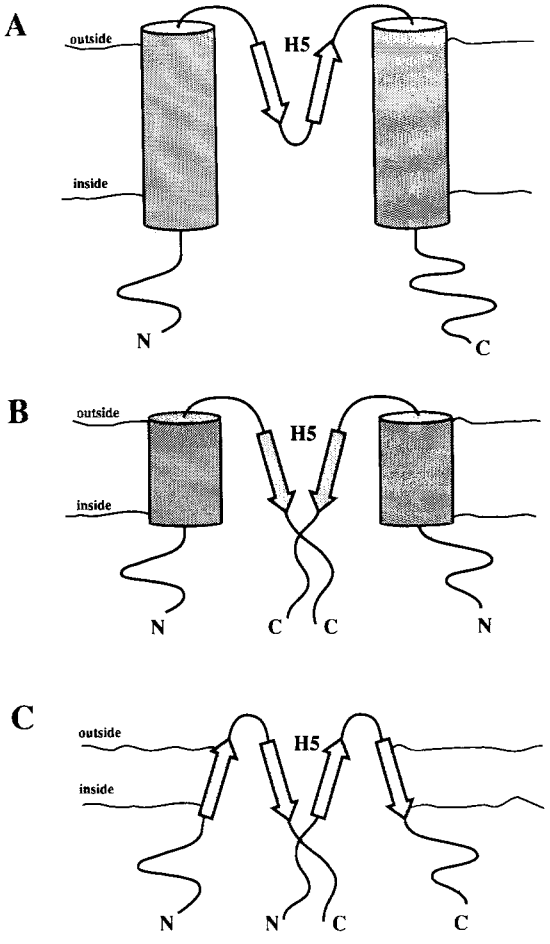

Fig. 4. Putative minimal folds for the K-channel pore. Assuming two β-strands for the pore-forming H5 segment (Durell & Guy, 1992) in voltage-gated K-channels, the pore in a tetrameric subunit assembly would be formed by an 8-stranded β-barrel. (*A*) Possible topology of subunits of ATP-regulated K-channel ROMK1 (Ho et al., 1993) and of inward rectifier IRK1 (Kubo et al., 1993). Each subunit contains two membrane spanning segments which hold the pore-forming H5 segment in place. Four subunits may form an active channel. *Outside:* extracellular; inside: intracellular side of membrane. Phospholipid bilayer is indicated by wavy lines. *N*: amino-terminus; *C*: carboxy-terminus. Transmembrane segments are diagrammed as cylinders; H5 segments as arrows to symbolize proposed β-strands. (*B*) Possible topology of subunits of minK-channels (Philipson & Miller, 1992). Each subunit might contain one membrane spanning segment and one half H5-like segment, forming one β-strand of a pore-forming β-barrel. In this case, two subunits are shown to form a structure which resembles the one of the other K-channels. Note that this model predicts that, in comparison to voltage-gated K-channels, twice as many subunits would be needed to form the minK-channel pore and that the β-barrel would be formed by a parallel β-sheet. (*C*) Alternative model of minK-channel subunits. Each subunit forms two β-strands entering and exiting the lipid bilayer from the cytoplasmic side. In this case, two subunits may form an antiparallel β-fold which is similar in structure to the one of the H5 β-fold of other K-channels. Note this model requires only four subunits to form an 8-stranded β-barrel as a K-channel pore.

drophobic, possibly membrane spanning segments for their activity. The ROMK1 and IRK1 K-channels may have what might be the minimum fold for forming K-channels: an S5-like segment, the H5 region, and an S6-like segment (Fig. 4). The probably cytoplasmic amino termini contain a sequence, which is reminiscent of an S4-type voltage sensor. The carboxy-terminus in the case of ROMK1 harbors a putative ATP-binding fold. These interesting K-channels pose a number of questions concerning their possible structure. One possibility is that they represent members of independent families of K-channels unrelated to *Shaker*-like voltage-gated K-channels. Alternatively, the channels may represent the more ancient design for forming K-channels. Possibly ROMK1 and IRK1 assemble as multimers in a comparable manner to *Shaker*-like K-channels, in which four H5 segments may be the essential contributor to lining the pore. This hypothesis implies that the additional hydrophobic segments (S1 to S3), found in the *Shaker* family of K-channels, may not be necessary for forming functional channels, but may subserve other functions.

MinK-channels are encoded by a small, 130 amino acid long polypeptide, with a single predicted hydrophobic segment of 23 amino acids. Since the amino- and carboxy-terminus of minK proteins are apparently cytoplasmic, the minK subunits should enter and exit the membrane from the cytoplasmic side (Philipson & Miller, 1992) (Fig. 4). MinK-channel subunits could fold and insert into the membrane in a comparable manner to the H5-region fold of the other K-channels.

The hypothetical folding of minK-channel subunits, as illustrated in Figure 4*B,C*, predicts that the assembly of two minK-channel subunits is equivalent to the structure of one subunit of 'standard' K-channel subunits shown in Fig. 4*A*. Thus, the assembly of minK-channels could also result in the formation of an 8-stranded β-barrel comparable in structure to the one of voltage-gated K-channels. It will be very interesting to find out whether nature has in fact used one type of folding pattern for forming

K-channel pores. Analysis of crystal structures of soluble proteins has shown that often seemingly different primary sequences of related enzymes (e.g., dehydrogenases) fold into very similar structures for binding analogous substrates. In vitro mutagenesis of K-channels in particular has carried us a long way in studying the structure and function of channel pores. But we are still far away from a clear picture of the structure of a pore and of an understanding of how a pore may function. In the future, structural data on ionic channels, obtained at high resolution, may reveal how K-channels create a pore and how different or similar they are in their folding pattern.

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