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Minireview

The viral potassium channel Kcv: structural and functional features

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Abstract **The chlorella virus PBCV-1 was the first virus found to encode a functional potassium channel protein (Kcv). Kcv is small (94 aa) and basically consists of the M1-P-M2 (membrane-pore-membrane) module typical of the pore regions of all known potassium channels. Kcv forms functional channels in three heterologous systems. This brief review discusses the gating, permeability and modulation properties of Kcv and compares them to the properties of bacterial and mammalian K^+ channels.**

Key words: K^+ channel; Kcv; PBCV-1; Chlorella virus

1. Introduction

Voltage- and ligand-gated potassium (K^+) channels control membrane potential in a variety of cell types in higher plants and animals [1]. These channels represent the result of tens of millions of years of evolution. K^+ Channels are also found in prokaryotes and lower eukaryotes and probably exist in all life forms. Kcv was the first K^+ channel protein found to be encoded by a virus [2]. Furthermore, Kcv represents the smallest and most primitive form of K^+ channels known so far. The 94 amino acid (aa) Kcv is encoded by the large dsDNA-containing *Paramecium bursaria* chlorella virus (PBCV-1) [3]. Kcv expression in heterologous systems [2,4,5] revealed properties similar to those of structurally more complex K^+ channels, including a moderate voltage dependence, selective block and modification of activity mediated by several intra- and extracellular factors. This combination of structural simplicity and functional complexity prompted more detailed structural studies of Kcv.

2. Structural implications from the aa sequence

Hydropathy analysis of the Kcv aa sequence reveals two putative transmembrane domains (M1 and M2) separated by 44 aa that contain the K^+ channel signature sequence TXXTXGFG [2]. In contrast to other K^+ channels, Kcv lacks cytoplasmic N- and C-termini, except for 12 aa at the N-terminal end. Therefore, Kcv is essentially comparable to the 'pore module' of all K^+ channels having M1-pore-M2 regions. The sequence of Kcv (after removal of the first 12 aa) was aligned with the pore module of KcsA, a pH-gated bacterial K^+ channel [6]. The structure of the pore of KcsA has been solved by crystallography [7] and consists of M1, the extracellular portion named 'turret', the pore helix, the selectivity filter and M2 (Fig. 1a). Kcv alignment with KcsA indicates 42% aa similarity and 19% aa identity; however, gaps occur in the turret and in the TM2 region. Also the Kcv TM2 domain is shorter than that of KcsA; importantly, this domain lacks a centrally located glycine (residue 99 of KcsA) which is conserved in most K^+ channels. This glycine has been proposed to have a specific role in channel gating [8]. Two structurally important aromatic aa are conserved in the pore helix of Kcv (residues 55 and 56). Crystallographic studies on KcsA indicate that these two residues are part of a structure that acts as a cuff to keep the pore open to the appropriate diameter for K^+ passage [7]. In Kcv, as well as in other K^+ channels (Herg, Kch, see [7]), the two tryptophan (WW) residues are replaced by the aromatic tyrosine (Y) and phenylalanine (F) residues.

Fig. 1b shows the comparison between the structure of KcsA and a hypothetical structure for Kcv derived from the KcsA coordinates. Although hypothetical, the graphic representation localizes regions of low homology between the two sequences, which are highlighted in red. The implications of these findings for Kcv channel function are discussed in the following sections.

One of the most remarkable feature of Kcv resides in the N- and C-termini. The cytoplasmic N-terminus, (^{1}MLVFSKFLTRTE^{12}) is extremely short but contains two positively charged aa (K^6, R^{10}) and a putative phosphorylation site (^{9}TRTE^{12}); the cytoplasmic C-terminus is completely absent, so that the C-terminus is part of TM2.

3. Properties of Kcv conductance in heterologous expression systems

Expression of Kcv in *Xenopus laevis* oocytes (Fig. 2) reveals that the protein forms an ionic channel conducting inward and outward currents. Kcv currents can be dissected into

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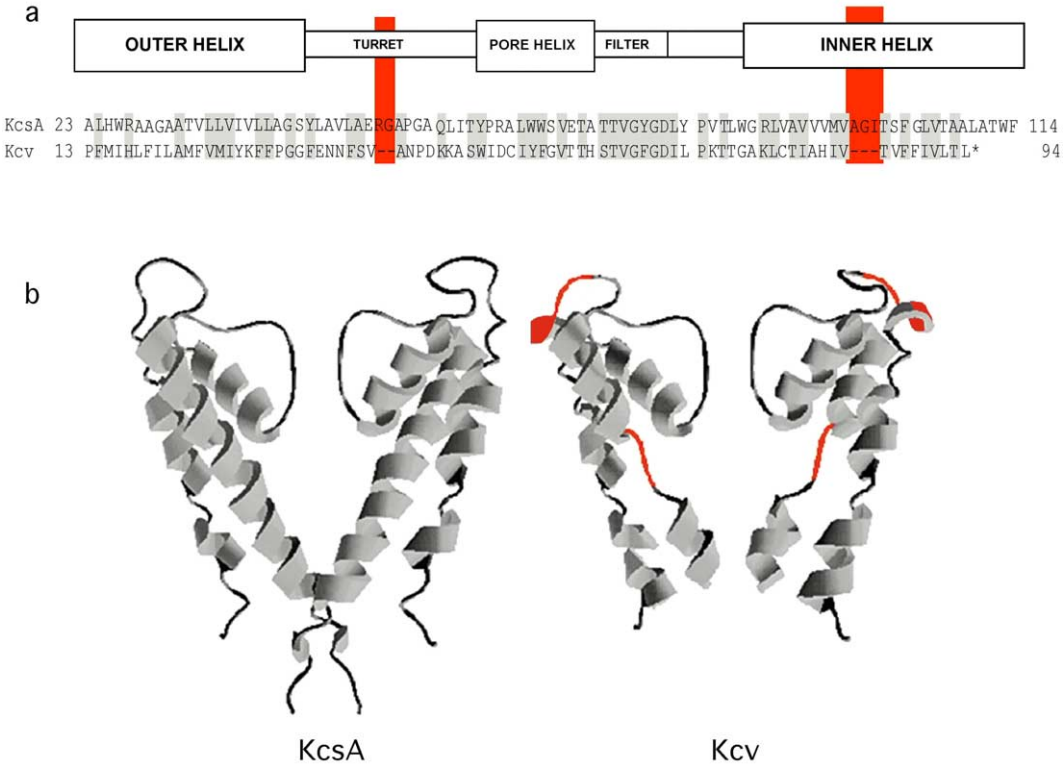

Fig. 1. a: Sequence alignment of the pore region of KcsA (aa 23–114) with Kcv (after removal of the first 12 aa, * indicates the end of Kcv sequence). Shown on top is the schematic representation of pore subdomains according to the structure of KcsA: outer helix (M1), turret, pore helix, filter, inner helix (M2). Conserved substitutions are shaded in gray. Sequence similarity was 42%. Red-shaded areas highlight regions of low homology between the two sequences, where gaps were introduced in the Kcv sequences. The glycine (G) 99 in the inner helix of KcsA is conserved among potassium channels and is involved in gating, see text. b: Structural model of KcsA and hypothetical model of Kcv obtained from KcsA by means of the alignment shown in a. Only two of the four subunits of the tetrameric channels are shown. Highlighted in red are the regions of low homology between Kcv and KcsA, in the turret and in the inner helix. The model was generated with KcsA as the reference structure by means of the Deep View Swiss-Pdb Viewer program (http://www.expasy.org/spdbv/) [22] linked to Swiss-Model, an automated homology modeling server.

two distinct kinetic components: an instantaneous component and a time-dependent component. The current/voltage relation of both components is linear over a broad voltage range and decreases progressively at extreme voltages. These results suggest that Kcv is slightly voltage sensitive.

Expression of foreign proteins in *Xenopus* oocytes can produce artifacts because the overproduction of a recombinant protein can cause the oocytes to upregulate endogenous channels [9]. A well-known example of this phenomenon is the expression of the minK protein in oocytes. This small protein was originally considered to be a miniature K^+ channel before it was discovered that the increase in K^+ conductance was due to an upregulation of endogenous outward rectifying K^+ channels [10]. Therefore, we conducted several control experiments to establish that the conductance seen in oocytes after injection of Kcv cRNA was due to a channel function of the Kcv protein. A mutant of Kcv was created in which the Phe^{66} in the selectivity filter was exchanged for Ala. K^+ channels with this aa substitution are known to be expressed in oocytes but are unable to conduct a current [11]. The Kcv mutant did not produce a conductance in *Xenopus* oocytes [2].

The Kcv protein was also expressed in two additional heterologous systems. Using a chimera of the Kcv with the green fluorescent protein (GFP), a Kcv-related conductance increase was detected in mammalian HEK293 [5] and Chinese hamster ovary (CHO) cells (Fig. 2). In addition, the expression and cellular localization of the chimera was monitored by confocal laser scanning microscopy. The fused Kcv protein was detected in the plasma membrane. However, the Kcv channel was not limited to the plasma membrane but was also present in the intracellular membranes. At present, it is unknown if these signals represent Kcv association with the cytoskeleton and/or with the endoplasmic reticulum.

Even though the Kcv protein did not accumulate exclusively in the plasma membrane, a distinct increase in plasma membrane current occurred in cells transfected with Kcv-GFP. This current shared several features with the Kcv current found in *Xenopus* oocytes; however, differences also occurred. The Kcv currents in mammalian cells and oocytes had the same selectivity for K^+ over Na^+ and a similar sensitivity to inhibition by Ba^{2+} and amantadine. Also the decrease of the instantaneous current at extreme negative and positive voltages was similar in the three expression systems. However, the time-dependent inward current, which is a typical component of the conductance in *Xenopus* oocytes, was rarely observed in mammalian cells. That is, only mammalian cells that expressed high levels of Kcv contained this time-dependent component. Detailed analysis of this component in HEK293 cells also indicated that the voltage dependency differs between the expression systems. In HEK 293 cells, the voltage

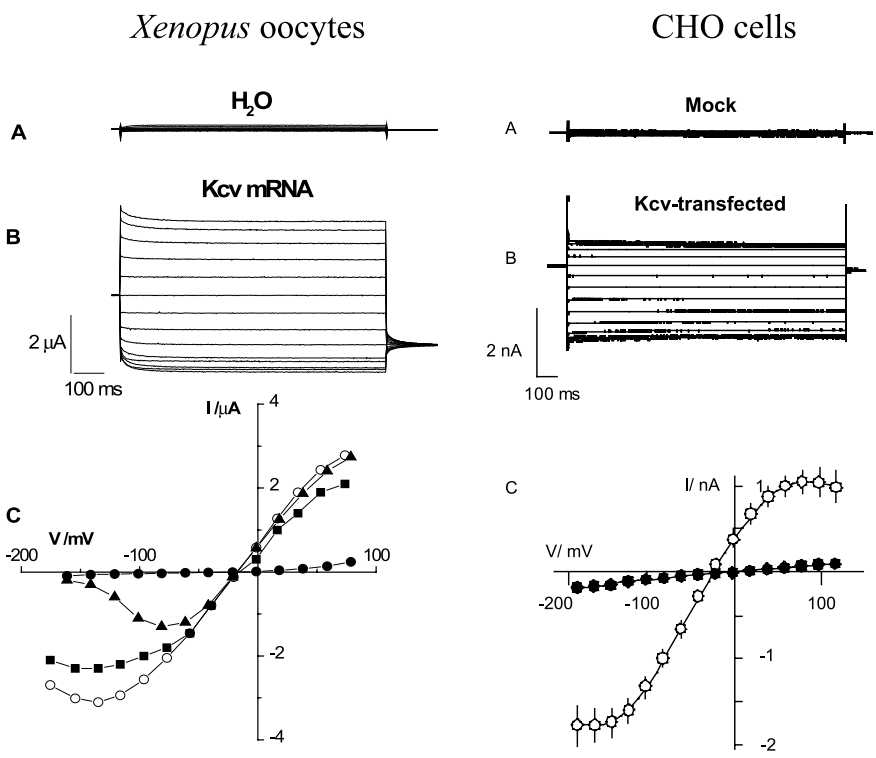

Fig. 2. Comparison of the currents recorded by heterologously expressing Kcv in *Xenopus* oocytes and CHO cells. *Xenopus* oocytes: voltage protocol from −180 mV to +80 mV, holding potential −20 mV, tails −80 mV, external solution contained 50 mM KCl. A: Currents measured by two-electrode voltage-clamp in one control oocyte (water injected). B: Currents measured in one oocyte injected with 50 ng of Kcv cRNA. C: Steady state current–voltage relation: (●) water injected and (○) Kcv injected. Also shown are two modalities of current inhibition: (▲) voltage-dependent inhibition of Kcv current by external 1 mM Sr^{++} and (■) voltage-independent inhibition of Kcv current by 10 mM external TEA CHO cells: voltage protocol from −180 mV to +120 mV, holding potential and tails 0 mV, external solution contained 30 mM KCl. A: Currents measured by patch-clamp in whole cell configuration from a control cell (mock transfection). B: Currents measured from a cell transfected with the chimeric construct Kcv-GFP. C: steady state current–voltage relation of: (●) control and (○) Kcv-GFP transfected cells. Data are averages of 10 cells.

for half-maximal activation was shifted to more positive potentials by about 60 mV as compared to oocytes.

Heterologous expression of the Kcv-GFP construct was also obtained in CHO cells. Although the general properties of the current were preserved, again the most prominent difference with oocyte expression was the apparent lack of the time-dependent component (Fig. 2).

Taken together these results demonstrate that Kcv expression in heterologous systems produces K^+ selective currents with basic properties which are independent of the expression system. Thus, Kcv currents are not due to an up regulation of endogenous channels but reflect the properties of the virus-encoded channel protein. As mentioned above, however, differences exist in the kinetic properties between the expression systems. Expression systems are known to affect the properties of other channels (e.g. [12]), but the reasons for these differences are unknown. In the case of Kcv the different properties of the channel could reflect different cellular environments or even differences in channel assembly. In this regard, the aforementioned shift of 60 mV in the activation curve of Kcv in mammalian cells relative to oocytes suggests that a 'cytoplasmic factor' might alter the range of activation of the current. It is also worth noting that compared to other K^+ channels, Kcv has short transmembrane domains It is therefore even possible that the thickness of the membrane in which the channel is inserted affects its performance.

4. Gating

Kcv channels show a linear conductance over a wide range of membrane voltages (roughly −60 to +60 mV). In this range the channel is always open and does not show any gating mechanism that closes the pore. In voltage- or ligand-gated channels, the main gate which mediates the transition from the closed to the open configuration is presumably located at the cytosolic end of the pore [8,13,14]. A comparison of the closed structure of the KcsA channel and the open structure of the MthK channel shows that a pronounced bend of the inner helix M2 in the MthK channel widens the cytosolic half of the pore, allowing the flow of ions [8]. The finding that Kcv does not appear to have a gate is consistent with the observation that Kcv's M2 is short when compared to that of KcsA. It also lacks a glycine in the center (see Fig. 1), i.e. an essential part of the proposed gating mechanism. Specifically, glycine is the 'gating hinge', a rather flexible position, where the bending of the M2 helix occurs to allow the pore to open [8].

Even though the Kcv structure is simple and presumably always in the open state, nevertheless Kcv currents exhibit distinct kinetic and voltage-dependent features when expressed in oocytes. Close examination of these Kcv currents reveals an instantaneous and a time-dependent components during voltage-clamp steps. Both components have an identi-

cal sensitivity to the inhibitor amantadine, implying that they reflect distinct kinetic states of the same channel [4]. The experimental data can be described formally by straightforward equations [4]. This analysis reveals that about 70% of the Kcv channels are always open at all voltages. At negative voltages the remaining ~30% of channels open in a time- and voltage-dependent manner reaching half-maximal activation at about −70 mV.

Another peculiarity of Kcv is that at both extreme positive and negative voltages the open Kcv channel conductance decreases. To examine the mechanism(s) responsible for the observed Kcv voltage dependence, the two charged aa in the 12 aa N-terminus were converted to alanine (mutant Kcv K6A R10A). However, this double mutation had no effect on the voltage dependence of the Kcv channel [4]. This indicates that these charged aa and the short lipophylic N-terminus in which they are inserted is not a membrane-embedded voltage sensor. We also tested the action of external divalent cations on the voltage dependence of the Kcv channel. A 10-fold reduction in external Ca^{2+} concentration increased the Kcv current about four-fold. This pronounced increase in current was observed with Ca^{2+} but not Mg^{2+}, and was voltage independent. These results indicate a Ca^{2+} selective, but voltage-independent mechanism, regulating channel conductance [4]. Taken together, these data indicate that the two charged aa in the N-terminus are not involved in voltage dependency. They also exclude a voltage-dependent block of the channel by divalent ions as the mechanism of voltage dependency. Hence, the mechanism of voltage dependency is unknown; the question will be addressed by future molecular modifications of the channel protein.

5. Selectivity

Potassium channels exhibit very similar ion permeability. The selectivity sequence is usually $K^+ = Rb^+ > Cs^+$, whereas permeability for the smallest alkali ions Na^+ and Li^+ is extremely low. [1].

Kcv is a K^+ selective channel and is much less permeable to sodium. Independent of the expression system a permeability ration P_{K^+}/P_{Na^+} of ~10 was determined [2,5]. The complete selectivity sequence for monovalent cations is $Rb^+ \geq K^+ > Cs^+ \gg Na^+ > Li^+$. Despite its very high permeability, external Rb^+ was found to strongly inhibit the inward conductance of Kcv in a voltage- and time-dependent manner. This inhibition is due to an inactivation phenomenon only observed at voltages more negative than −50 mV (Kang et al., manuscript in preparation).

6. Pharmacology

Kcv channels are blocked by external Ba^{2+}, a known inhibitor of K^+ channels, with a K_d at 0 mV of 0.6 mM [2]. The block is strongly voltage dependent and fully reversible. Sr^{++} blocks Kcv currents with similar modalities but with lower affinity (Fig. 2), while Mg^{2+} does not affect Kcv current to any extent [4]. Kcv channels are only slightly sensitive to external Cs^+. Kcv inward current is inhibited by 10 mM Cs^+ by only 15–20%. Low affinity inhibition occurs also with the K^+ channel blocker TEA, which inhibits at 10 mM Kcv conductance by only 30–40% at all voltages (Fig. 2). As mentioned before, Kcv is sensitive to amantadine, a well-known anti-viral drug, which blocks the influenza virus channel M2 at micromolar concentrations [15]. In contrast to M2, Kcv shows a lower affinity for amantadine. A K_d of 0.8 mM (at −140 mV) was measured for Kcv expressed in oocytes as well as in HEK293 cells in which amantadine inhibited Kcv in a voltage-independent manner [4]. Inhibition by amantadine with a similar low affinity, in the mM range, has also been reported for other K^+ channels [16] suggesting that at high concentrations the cyclic amine is a general inhibitor of K^+ channels.

Structural conservation in prokaryotic and eukaryotic potassium channel pores has been established by using scorpion toxins, which bind specifically to the external side of the channel pore and cause inhibition [17]. Therefore we tested Kcv for inhibition by Charybdotoxin and Agytoxin-2, two antagonists that specifically block wild-type Shaker and mutant KcsA channels [17–19]. Kcv was not affected by any of these toxins. In fact this result is not surprising because the aa similarity between Kcv and KcsA in the turret region is low and this region exposes most of the toxin binding sites [7,17].

7. Modulation

Preliminary experiments indicate that a physiologically relevant modulatory mechanism occurs in Kcv. Membrane-permeable protein kinase inhibitors (staurosporine, H89, A3) inhibit Kcv conductance in *Xenopus* oocytes. A prosite scan of the channel sequence identified two putative CK2 phosphorylation sites. One of them (^{9}TRTE^{12}) is located in the cytoplasmic N-terminus. This site was also recognized as a putative site of interaction with 14.3.3 proteins, a class of modulatory proteins known to interact with K^+ channels in plant and animal cells [20,21]. Mutations performed in the N-terminal putative phosphorylation sites (T9A) did not change the properties of the Kcv channel, nor did they affect its sensitivity to protein kinase inhibitors. However, deletion of the first 14 aa at the N-terminus resulted in a non-conducting channel [5]. This result suggests the short N-terminus is a possible modulatory region of the channel conductance.

8. Conclusions

Structural and functional features of Kcv indicate that this miniature K^+ channel displays a wide array of common properties with more complex animal and plant channels, and can therefore be considered as a useful tool in channel biophysics.

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