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pH-dependent regulation of a potassium channel protein encoded by a *Chlorella* virus PBCV-1

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ABSTRACT In our project we studied a K^+ channel, Kcv, encoded by a large *Chlorella* virus, PBCV-1. We investigated the pH dependency of the channel. The data reveal a weak dependency of the channel conductance on extracellular and intracellular pH.

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Ion channels provide one of the fundamental means of transport across the membrane. Recently, molecular biology techniques have been employed to understand structure-function relations of ion channel proteins. These techniques enable to perform subtle changes in the structure of channel proteins, and monitor subsequent physiological changes with electrophysiological techniques. In our project we studied the small K^+ channel, Kcv, encoded by the large phytophathological virus, PBCV-1. Expression of the channel in *Xenopus* oocytes showed that Kcv functions as an ion channel and exhibits definite K^+ currents in voltage clamp assays. Furthermore, it has been shown that this channel is essential to the virus life cycle (Plugge et al. 2000). Structural data and phylogenetic analysis suggest that Kcv – the first functional ion channel identified in a virus – represents a very primitive K^+ channel. Due to its relatively small size, Kcv is an ideal candidate for investigating structure-function relations and for identifying the basic structural components essential for channel function. Our study focused on the pH dependent regulation of Kcv supposedly effected by a putative pH sensor in the pore region of channel.

Materials and Methods

Human embryo kidney cells (HEK293) were maintained at 37 ºC, 4% CO_2 in DMEM containing 10% FCS, 10 U/ml penicillin and streptomycin. The cells were passaged when they approached confluency by trypsinising with 0.25 % trypsin/EDTA and seeding into fresh medium.

pEGFP-N2 plasmids containing the Kcv and EGFP genes were transfected in HEK 293 cells using the calcium phosphate precipitation method. The Petri dishes with transfected cells were mounted on a fluorescent inverted microscope and constantly perfused with bath solution. Successfully transfected cells were visually identified by their green fluorescence, and EGFP negative cells from the same batch were used as controls.

The bath solution typically contained (mM): Choline Cl, 110; $CaCl_2$, 1.8; $MgCl_2$, 1.0; Hepes, 5; KCl, 30; and was set to a pH of 7.4 using NaOH. Throughout the measurements the pH of the external solution was changed to 5.5, 6.5, 7, and 8.5 by a liquid perfusion system.

Whole-cell pipettes were filled with an intracellular-like solution containing (mM): NaCl, 10; KCl, 130; EGTA, 1.0, Hepes-KOH, 5; $MgCl_2$, 0.5; ATP (Na salt), 2; GTP (Na salt), 0.1; Phosphocreatine, 5 (pH = 7.2).

Potassium currents were recorded using standard patch clamp whole cell recordings. Patch pipettes were made of borosilicate glass capillaries (2-5 MW; Kimax-51, Kimble, Toledo, OH). Currents were recorded using a List EPC 7 patch-clamp amplifier (List Medical, Darmstadt, Germany). Currents were filtered at 3 kHz sampled on computer under control of pClamp 5 hard/software (Axon Instruments, USA).

Results and Discussion

Whole cell conductances of transfected and non-transfected HEK293 cells were monitored by applying a standard voltage protocol. Cells were clamped for 1.5 s from a holding potential of –20 mV, to test voltages between +60 mV to -120 mV, followed by a 200 ms pulse to –80 mV. In this assay, cells transfected with Kcv:GFP revealed a marked difference in the whole cell currents compared to untransfected control HEK293 cells (Fig. 1A). This allowed a clear cut separation of the Kcv related currents from the background currents.

Exposing non transfected cells to bath solutions with pH values ranging from 5.5 to 8.5 had no perceivable effect on the background conductance (data not shown) revealing a pH insensitivity of the background currents.

On the other hand, a test of the Kcv:GFP conductance over a range of pH 5.5 to pH 8.5 revealed a slight pH dependency in the sense that the conductance increased by about 20% between pH 6 and 8.5. For this analysis the conductance was measured for each cell initially at pH 7.4 as reference value. Subsequently, cells were exposed to solutions with the respective test pH and data were normalized to the values of pH 7.4. Figure 1B illustrates relative pH dependency of the Kcv:GFP current at -120 mV. Fitting this curve with a Hill-function yields a pK value of 7.15 and a Hill coefficient of 1.8. Notably, the pK value is

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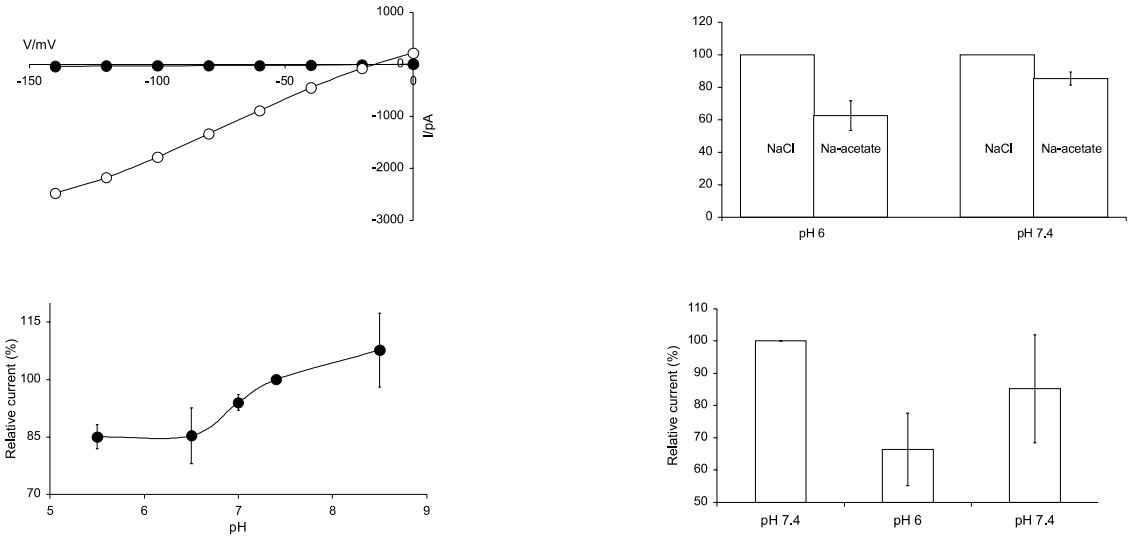

Figure 1. pH-dependency of the Kcv channel. For further details see text.

in the range the pK of titratable Cys (pK 8.3) and His (pK 6) side chains. It may be speculated that one of these amino acids is mediating the pH dependency of the channel protein.

To test the effect of reducing the cytoplasmic pH, we compared the effect of treating cells with both 30 mM NaCl and 30 mM Na-acetate respectively. The rationale behind these experiments was that at pH 6 – the pH value of both solutions – acetate is partially protonated and diffuses freely into the cell. There it dissociates and releases its H^+, hence decreasing the intracellular pH. Following this strategy we found that acidification of the cytoplasm entailed a K^+ current decrease (to 63 ± 9 %, n = 7), which was completely reversible after removing the acetate from the external solution. In control experiments cells were given the same treatment at pH 7.4. Under these conditions the impact of proton loading and hence cytoplasmic acidification should be smaller by more than an order of magnitude. However, in these experiments we also found that the Kcv:GFP conductance was still reduced, albeit the current decreased to only 85.5 ± 4 %, n=3. The data suggest that at least 20% of the Kcv conduct-ance is inhibited specifically by cytoplasmic acidification (Fig. 1C).

Figure 1D summarizes experiments in which the cells where bathed in a solution with 30 mM Na-acetate at either pH 7.4 or 6. Currents were measured in the whole cell configuration upon clamping the membrane from a holding potential of -20 mV to -120 mV test voltage. Switching from pH = 7.4 to pH = 6 resulted in a reduced K^+ inward current (66 ± 11 %, n = 6), while the return to pH = 7.4 recovered the current at the same voltage conditions (85 ± 16 %, n = 6).

In summary, the results indicate that Kcv channel is weakly pH-dependent. Future structural analysis may uncover the role of titratable amino acids in the channel mediating this dependency.

Acknowledgments

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Reference

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