Biochimica et Biophysica Acta 1838 (2014) 1096–1103

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

# Viral potassium channels as a robust model system for studies of membrane–protein interaction☆,☆☆

Christian J. Braun [a], Christine Lachnit [a], Patrick Becker [a], Leonhard M. Henkes [b], Cristina Arrigoni [c], Stefan M. Kast [b], Anna Moroni [c,d], Gerhard Thiel [a], Indra Schroeder [a,*]

[a] Membrane Biophysics, Technical University of Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany
[b] Physikalische Chemie III, Technische Universität Dortmund, Otto-Hahn-Str. 6, 44227 Dortmund, Germany
[c] Department of Biosciences, University of Milan, Via Celoria 26, 20133 Milano, Italy
[d] CNR-IBF, Via Celoria 26, 20133 Milano, Italy

ARTICLE INFO

*Article history:*
Received 25 April 2013
Received in revised form 31 May 2013
Accepted 8 June 2013
Available online 17 June 2013

*Keywords:*
Black lipid membrane
Cell-free protein expression
Membrane–protein interaction
Planar patch clamp
Single-channel measurement
Viral potassium channel

ABSTRACT

The viral channel $Kcv_{NTS}$ belongs to the smallest $K^+$ channels known so far. A monomer of a functional homotetramer contains only 82 amino acids. As a consequence of the small size the protein is almost fully submerged into the membrane. This suggests that the channel is presumably sensitive to its lipid environment. Here we perform a comparative analysis for the function of the channel protein embedded in three different membrane environments. 1. Single-channel currents of $Kcv_{NTS}$ were recorded with the patch clamp method on the plasma membrane of HEK293 cells. 2. They were also measured after reconstitution of recombinant channel protein into classical planar lipid bilayers and 3. into horizontal bilayers derived from giant unilamellar vesicles (GUVs). The recombinant channel protein was either expressed and purified from *Pichia pastoris* or from a cell-free expression system; for the latter a new approach with nanolipoprotein particles was used. The data show that single-channel activity can be recorded under all experimental conditions. The main functional features of the channel like a large single-channel conductance (80 pS), high open-probability (>50%) and the approximate duration of open and closed dwell times are maintained in all experimental systems. An apparent difference between the approaches was only observed with respect to the unitary conductance, which was ca. 35% lower in HEK293 cells than in the other systems. The reason for this might be explained by the fact that the channel is tagged by GFP when expressed in HEK293 cells. Collectively the data demonstrate that the small viral channel exhibits a robust function in different experimental systems. This justifies an extrapolation of functional data from these systems to the potential performance of the channel in the virus/host interaction. This article is part of a Special Issue entitled: Viral Membrane Proteins—Channels for Cellular Networking.



## 1. Introduction

Potassium channels are membrane proteins, which catalyze the flux of $K^+$ ions in a selective and regulated (gated) manner across membranes. Because of their high transport capacity, the activity of single $K^+$ channels can be measured at high temporal resolution with various electrophysiological methods. This detailed functional information is paralleled by explicit knowledge on the molecular architecture of $K^+$ channels, which is available from crystal structures [1–3] and MD simulations [4,5]. The combination of these high-resolution data is now frequently used to correlate structural properties with functional features [6,7].

In this context, however, it occurs that for a full understanding of ion channel function the membrane, in which the protein is embedded, has to be considered also. Crystal structures for example reveal the binding of anionic phospholipids to specific pockets in the KcsA protein [8]. Complementary functional studies underscore the role of these interactions for channel function [9–11]. Structural data furthermore suggest that the architecture and orientation of transmembrane domains of membrane proteins are designed in such a way that they avoid a thermodynamically unfavorable hydrophobic mismatch between protein and lipid bilayer [12–14]. This predicts that the thickness of the membrane, which can vary in the plasma membrane within microscopic domains [15], may affect the structure of a channel and as a consequence also its activity. Functional studies in this context have indeed shown that the conductance of the BK channel is depending on the thickness of the lipid bilayer in which it is inserted [16]. But the

*Abbreviations:* BLM, black lipid membrane; DPhPC, 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine; GUV, giant unilamellar vesicle

☆ This article is part of a Special Issue entitled: Viral Membrane Proteins—Channels for Cellular Networking.

☆☆ Funding support: The investigations were supported by Cariplo grant 2009-3519 (AM, IS), PRIN 2010CSJX4F (AM), Deutsche Forschungsgemeinschaft and by Loewe Cluster Soft-Control (GT, IS).

* Corresponding author. Tel.: +49 6151 16 3503.
*E-mail address:* schroeder@bio.tu-darmstadt.de (I. Schroeder).

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http://dx.doi.org/10.1016/j.bbamem.2013.06.010

membrane-spanning domains of the proteins seem to be not the only ones involved in lipid-channel interaction. A recent study suggests that the M0 helix of KcsA, which is lying on the cytosolic membrane interface, performs a "barrel roll" movement upon channel opening; this event seems to be dependent on the presence of anionic lipids in the inner membrane leaflet [17].

The activity of ion channels is generally recorded with the patch clamp method [18] in membranes from living cells, which express a channel of interest in a homologous or heterologous manner. In this case, the composition of the membrane in which the channel functions, is largely unknown and not under the control of the experimenter. In such a system, there is also little control over the arrangement of the channel proteins in clusters, their interaction with endogenous proteins or their insertion into micro-domains like lipid rafts. A reduced system, which offers more control over the environment of a channel protein, is provided by the planar lipid bilayer technique [19,20]. With this method, the lipid bilayer can be chosen and modified by the experimenter on demand. This offers the opportunity to reconstitute a purified protein into a pure and defined bilayer. The problem with this approach is that bilayers may under certain circumstances also generate lipid pores [21] so that bilayer recordings with reconstituted proteins are sometimes challenged as artifacts [22]. Another source of artifacts in bilayer recordings is related to the isolation procedure of the channel proteins. Protein produced recombinantly or isolated from cells may be contaminated with endogenous channel proteins from the expression system [23,24]. A further disadvantage of the bilayer technique is that some protocols require a solvent like decane for building the bilayer [20]. In the procedure some solvent may stay in the bilayer and modify the membrane in an unpredictable fashion. Another technique generates planar lipid bilayers from giant unilamellar vesicles (GUVs) [25]. This approach does not require any solvents; but in this protocol, usually 10% cholesterol is used for the fabrication of stable membranes.

In this work, we compare the basic single-channel properties of a model $K^+$ channel in a variety of different recording systems. The small viral $K^+$ channel $Kcv_{NTS}$ is encoded by a *Chlorella* virus isolated from an alkaline lake in Nebraska [26]. $Kcv_{NTS}$ is structurally very similar to $Kcv_{ATCV-1}$ from *Acanthocystis turfacea Chlorella* virus-1 (Fig. 1). The latter is the prototype of the most recently isolated *Chlorella* virus group, which infects SAG type *chlorella* cells [27]. Previous studies have already shown that both viral proteins are functional $K^+$ channels [26,28]. The interesting aspect in the context of the present work is that $Kcv_{ATCV-1}$ and its orthologs are miniature indeed [29]. The four monomers, which form the functional tetramer, each contain only 82 amino acids. As we will demonstrate below by molecular dynamics (MD) simulations this small channel is quasi fully immersed in the lipid bilayer. We consider this an ideal model system for studying the relevance of protein/bilayer interactions. The environment of the protein is dominated by the lipid bilayer; extracellular or cytosolic domains can, if any, only play a minor role for channel function.

The systematic examination of viral $K^+$ channel function in different experimental conditions is important for a proper understanding of their role in the virus/host system. The current knowledge is that the great majority of *chlorella* viruses (39 over 41), which were sequenced so far, contain genes for small $K^+$ channels [30]. Expression studies show that the channel protein is produced as a late gene in the infected host cell [31]. Recent experiments confirm with the help of a monoclonal antibody that the prototype channel Kcv from virus PBCV-1 is indeed present in the mature virion; it is presumably located in the inner membrane of the virus particle [32]. A bulk of circumstantial data suggests that the viral channel has a crucial role during early infection. Measurements of the membrane potential in the host *Chlorella* NC64A, a unicellular green alga, have shown that the cells depolarize within the first few minutes of virus infection [33]. This depolarization is most likely caused by an insertion of few individual viral $K^+$ channels into the plasma membrane of the host during fusion of virus and host cell membrane [34]. Experimental support for this hypothesis comes from the observation that the same blockers, which inhibit the viral channel in heterologous systems or after reconstitution in planar lipid bilayers, also block the host depolarization [33,34]. The crucial role of the viral channel in host infection is further underscored by experiments, which show that the sensitivity of host depolarization to channel blockers reflects the distinct inhibitor sensitivity of $K^+$ channel orthologs from different viruses [33]. It was further found that a block of viral $K^+$ channel activity and the consequent inhibition of the host depolarization prevent infection. The latter could be attributed to a block of DNA ejection from the virus particle into the host [34]. A plausible explanation for this scenario is that the depolarization of the host cell, which is initiated by the viral channel, causes an efflux of $K^+$ salts and consequently water from the host cells [35]. This lowers the high internal pressure of the host cell and makes it easier for the virus to transfer its large dsDNA genome into the host. This interpretation of viral channel function during infection is based on extrapolations of functional data from heterologous expression systems and from reconstituting the viral channel protein in planar lipid bilayers. Because of the small size of the channels and the aforementioned potential dependency of channel function on the lipid bilayer it was so far not known whether these extrapolations are indeed valid.

The present data now show that conductance and gating properties of the channel are robust and observable in conventional patch clamp recordings and in different planar lipid bilayer experiments. With the exception of a smaller conductance and a slightly lower open-probability in the mammalian cell system, the basic functional features of the channel are maintained in all experimental approaches. There is little difference on whether the protein is synthesized by a mammalian cell, expressed and purified from yeast or produced in a cell-free system. The results of these experiments stress that functional data from different approaches can be used to draw conclusions on structure/function correlates in this miniature channel; the experimental data are also sufficiently general and allow an extrapolation of channel function in the virus/host system.


**Fig. 1.** Amino acid sequence alignment of $Kcv_{ATCV-1}$ and $Kcv_{NTS}$. Exchanges are marked in gray, the signature potassium filter sequence is underlined. The predicted location of the two transmembrane helices TM1 and TM2 of $Kcv_{ATCV-1}$ was taken from [28].

## 2. Methods

### 2.1. Protein expression in Pichia pastoris

The ORF encoding the $Kcv_{NTS}$ gene was subcloned into a modified *P. pastoris* expression vector pPICZ A (Invitrogen) containing a Kozak consensus sequence, a His7 tag, a proteolytic site for the H3C protease and a LIC (ligation independent cloning) site on the N-terminus of the protein sequence. *P. pastoris* cells (SMD 1163 strain) were transformed with 3 μg of the PmeI linearized construct by using the *Pichia* Easy Comp™ kit as described by the manufacturer (Invitrogen, Carlsbad, CA, USA). Positive colonies were selected from YPDS (10 g/l bacto yeast, 20 g/l bacto peptone and 20 g/l dextrose) agar plates containing 50 μg/ml zeocin. Single-colony starting cultures were grown in BMGYH medium [see the *Pichia* expression kit manual (Invitrogen)] at 30 °C, 300 rpm for 36 h. After centrifugation at 3000 *g* for 10 min at 4 °C, the pellet was resuspended to a D600 of 4 in BMMH medium [see the *Pichia* expression kit manual (Invitrogen)] and grown at 30 °C, 300 rpm for 24 h. Each gram of cells was suspended in a 1:20 ratio of breaking buffer as in Gazzarrini et al. [28]. The presence of the overexpressed protein at the correct molecular weight was verified on SDS-PAGE and Western blot. Cells were broken with a Cell Disruptor (TS Series