Biochimica et Biophysica Acta 1838 (2014) 1322–1331

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Voltage-gated ion channel modulation by lipids: Insights from molecular dynamics simulations

Marina A. Kasimova [a,b], Mounir Tarek [a,c,*], Alexey K. Shaytan [b], Konstantin V. Shaitan [b], Lucie Delemotte [d,**]

[a] Université de Lorraine, SRSMC, UMR 7565, Vandoeuvre-lès-Nancy F-54500, France
[b] Lomonosov Moscow State University, Moscow 119991, Russian Federation
[c] CNRS, SRSMC, UMR 7565, Vandoeuvre-lès-Nancy F-54500, France
[d] Institute of Computational and Molecular Science, Temple University, Philadelphia, PA 19122, USA

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form 17 January 2014
Accepted 24 January 2014
Available online 8 February 2014

Keywords:
Sphingomyelin
Ceramide
PIP2
Kv1.2
Shaker

ABSTRACT

Cells commonly use lipids to modulate the function of ion channels. The lipid content influences the amplitude of the ionic current and changes the probability of voltage-gated ion channels being in the active or in the resting states. Experimental findings inferred from a variety of techniques and molecular dynamics studies have revealed a direct interaction between the lipid headgroups and the ion channel residues, suggesting an influence on the ion channel function. On the other hand the alteration of the lipids may in principle modify the overall electrostatic environment of the channel, and hence the transmembrane potential, leading to an indirect modulation, i.e. a global effect. Here we have investigated the structural and dynamical properties of the voltage-gated potassium channel Kv1.2 embedded in bilayers with modified upper or lower leaflet compositions corresponding to realistic biological scenarios: the first relates to the effects of sphingomyelinase, an enzyme that modifies the composition of lipids of the outer membrane leaflets, and the second to the effect of the presence of a small fraction of PIP2, a highly negatively charged lipid known to modulate voltage-gated channel function. Our molecular dynamics simulations do not enable to exclude the global effect mechanism in the former case. For the latter, however, it is shown that local interactions between the ion channel and the lipid headgroups are key-elements of the modulation.

1. Introduction

Voltage-gated potassium channels (VGKCs) are transmembrane (TM) proteins that enable the rapid and coordinated conduction of potassium ions across the cell membrane upon alteration of the TM potential. These channels are homotetramers, each monomer spanning the membrane six times (S1 to S6). The central pore, formed by the assembly of S5 and S6, allows potassium ions to flow. The S1–S4 helix bundles constitute the peripheral voltage-sensor domains (VSDs), which sense the changes in the TM potential and trigger the conformational change responsible for the gating, i.e. opening and closing of the pore. S4 of the VSD contains 4 to 7 positively charged amino acids that sense the electric field and move in response to it. Under depolarizing conditions, S4 transfers upwards (UP), resulting in the channel's activation. When the membrane is hyperpolarized, the electric field causes S4 to move outwards (DOWN), leading to the channel's deactivation and pore closure [1–5].

1.1. Interaction between VGKCs and lipid content

According to the crystal structures of Kv1.2 [6], a mammalian Shaker-like channel, and the Kv1.2/2.1 paddle chimera [7], the VSDs are embedded in the membrane with S4 being mostly shielded away from the lipids. For these channels, S4 carries six positively charged amino acids (R294, R297, R300, R303, R309 and K306 in Kv1.2, called hereafter R1, R2, R3, R4, R6 and K5). Molecular dynamics (MD) simulations of Kv1.2 and Kv1.2/2.1 embedded in lipid bilayers have shown that for the resolved structures, the top gating charges R1 and R2 come in interaction with the lipid headgroups, making stable electrostatic interactions with their negatively charged phosphates. Prior to the crystal structure determination, using site-directed spin labeling and electron paramagnetic resonance spectroscopy, Cuello et al. showed that the S4 segment of the bacterial VGKC KvAP is located at the protein/lipid interface [8]. The same result was obtained by Lee and MacKinnon, studying the action mechanism of a voltage-sensor toxin, VSTX1, from the Chilean Rose Tarantula [9]. Further MD simulations have reported that the positive residues of S4 interact with the headgroups of the lipids. When the activated open state of Kv1.2 was relaxed in a zwitterionic membrane environment, S4 in its 'UP' state position provided the opportunity for the top positive residues (R1 and R2) to interact with the negative charges of the lipid phosphate groups from the outer membrane leaflet [10–15]. Models of the resting/closed state conformation of

* Correspondence to: M. Tarek, Université de Lorraine, SRSMC, UMR 7565, Vandoeuvre-lès-Nancy F-54500, France. Tel.: +33 383 68 43 74.
** Corresponding author. Tel.: +1 215 204 4216.
E-mail addresses: marina.kasimova@univ-lorraine.fr (M.A. Kasimova), mounir.tarek@univ-lorraine.fr (M. Tarek), shaytan_ak@mail.bio.msu.ru (A.K. Shaytan), k.v.shaitan@molsim.org (K.V. Shaitan), lucie.delemotte@temple.edu (L. Delemotte).

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http://dx.doi.org/10.1016/j.bbamem.2014.01.024

the channel embedded in a model membrane have also shown that the 'DOWN' state position of the S4 helix favors the interaction of the bottom positive residues (R4, K5 and R6) with the headgroups of the lipids from the inner membrane leaflet [16,17]. In the modeling studies that uncovered the entire deactivation pathway, it was found that the top and bottom charges of S4 may interact respectively and simultaneously with the headgroups of the inner and outer membrane leaflets in given intermediate states [4,18–20]. Further experiments demonstrate the important influence of lipid headgroups on VGKC function: reconstitution of a bacterial VGKC KvAP in a positively charged bilayer abolishes the function of the channel [21]. An insight into the molecular level process was provided using MD simulations [22].

1.2. Modulation of VGKCs by the outer membrane leaflet composition

Several experimental findings confirm the fact that the lipid environment may modulate the function of VGKCs. The activation of Shaker is suppressed if the channel is reconstituted in a positively charged bilayer [21]. Also, the removal of the lipid headgroup by some enzymes results in severe modification of the VGKC function through the perturbation of the VSD activation/deactivation [13–15]. In cells, VGKCs are embedded in a complex membrane that is composed of different types of lipids and that is most of the time asymmetrical. Sphingomyelin, a zwitterionic phospholipid, is mainly found in the outer leaflet where it can represent up to 15% of the lipid content in mammalian cells [23]. Enzymes called sphingomyelinases target the headgroup of the sphingomyelin and cut it partly or entirely. Sphingomyelinase D removes the choline group from the lipid, yielding the negatively charged ceramide-1-phosphate and favoring thereby the activated conformation of VGKCs. Another enzyme called sphingomyelinase C removes the entire headgroup, yielding ceramide, a polar lipid that bears no phosphate group to anchor R1 and R2. This causes the closed state of the channel to be preponderant [23–25].

1.3. Modulation of VGKCs by the inner membrane leaflet

Other examples of lipid modulation are known. Phosphatidylinositol-(4,5)-bisphosphate (PIP2) is a negatively charged phospholipid from the inner leaflet of the plasma membrane that was shown to modulate the function of several ion channels. Functional and structural studies have revealed that PIP2 has a major impact on the stabilization of the open state of several potassium channels including KCNQ, hERG, Kir, HCN and TRP channels [26–36], despite its very low concentration in cells (~1%) [37]. The same effect was later found for the channels from the Shaker family [38,39], causing a gain-of-function effect, i.e. an increase in the ionic current. In the same study, a contradictory loss-of-function effect was also shown, manifested by the right shift of the G/V and Q/V curves.

Despite the importance of the lipid modulation in a variety of biological processes, the molecular mechanisms involved still remain elusive. The key stimulus of VGKC activation is changing the TM potential. However several pieces of evidences suggest that mechanical stress, i.e. membrane stretching, also modulates VGKC functions [40–42]. Changes in lipid composition might have a global effect related to membrane lateral tension. This scenario has been discarded in the systems studied here for two main reasons: (1) in the case of sphingomyelin and its products, Lu and collaborators have demonstrated that SMase D effect vanishes after removing charges in S4, supporting the notion that SMase D modification of sphingomyelin molecules alters the electrostatic environment of the VSD [23]; and (2) in the case of PIP2, the concentration of the charged lipids is too low (~1%) [37] to consider an impact on elastic properties of membrane. Accordingly we focus here on elucidating the electrostatic effect of lipid alteration.

To explain the lipid electrostatic effects on VGKCs, two hypotheses may be put forward: 1) altering the lipid headgroups could lead to a modification of the TM potential or a reshaping of the electric field profile through the VSD, an effect that we will call "global"; and 2) it may also change the interactions between the lipid headgroups and the S4 basic residues of the channel locally, an effect that we will therefore refer to as "local". In order to discriminate between the two mechanisms, we resort to atomistic MD simulations.

Specifically, we study the structural and dynamical properties of various states (activated, resting and intermediate) of the voltage-dependent potassium channel Kv1.2 in different lipid environments: in a broadly used zwitterionic bilayer (palmitoyl-oleyl-phosphatidylcholine), in a bilayer with a modified outer leaflet (sphingomyelin (SM) and its products, negatively charged ceramide-1-phosphate (C1P) and polar ceramide (Cer)) and in a bilayer with a modified inner leaflet (PIP2). SM can make up to 15% of the lipid content in specific cells and be involved in a global effect as well as in specific interactions. To investigate thoroughly the effect of altering SM to its products on the TM potential, we have started by studying bare asymmetric bilayer. We have found that going from zwitterionic SM to negatively charged ceramide-1-phosphate (C1P) and to polar ceramide (Cer) does indeed result in a rearrangement of the different components of the system. For C1P, specifically, this results in a noticeable change of the overall transmembrane potential, i.e. when the lipid headgroup nature changes from zwitterionic to charged, hinting to a potential global electrostatic effect. PIP2 lipids, on the other hand, represent only a small fraction of bottom membrane leaflet (~1%) [37] enabling us to rule out a global effect immediately. We have investigated therefore the existence of specific binding pockets for the headgroups of these lipids and identify the specific residues involved. Thus, in this case, we conclude that the specific electrostatic regulation mechanism dictates channel function.

2. Methods

2.1. Preparation of the systems

2.1.1. Bare bilayers

Three bare lipid bilayer systems were built to probe the global effect of lipid modification. All contained 100% palmitoyl-oleyl-phosphatidylcholine (POPC) in the bottom leaflet and 100% of a sphingomyelin-derivative with a palmitoyl-oleyl chain in the upper one: 1— sphingomyelin (SM), 2— ceramide-1-phosphate (C1P) and 3— ceramide (Cer). These bilayers were solvated with a 150 mM NaCl solution. The composition of the systems is presented in Table 1.

2.1.2. Kv1.2 embedded in different bilayers

Systems consisting of Kv1.2 in its open [6], intermediate and closed states [18] were embedded in five different bilayers: 1 — a symmetric POPC bilayer, the entire system was extracted from the previous simulations (see [18], for details); 2, 3, 4 — three sphingomyelin-derivative asymmetric bilayers (SM, C1P and Cer); and 5 — a POPC bilayer in which the inner membrane ring of lipids surrounding the channel was replaced by PIP2.

The channel's conformations were inserted in pre-equilibrated asymmetrical bilayers (SM/POPC, C1P/POPC and Cer/POPC). In order to avoid repulsive contacts, the lipid and water molecules less than 1 Å away from the Kv1.2 were deleted. The systems containing PIP2 ring in the lower bilayer leaflet were constructed using packmol [43]. We have considered a tolerance of 2.0 Å as the minimum distance between packed molecules. All the systems were solvated with water and ionized to 150 mM KCl concentration. For the details of the systems' composition, see Table 2.

2.1.3. Force field parameters

The CHARMM22 force field with CMAP correction was used to build protein topology [44], together with the TIP3P model for water [45]. CHARMM27 and its compatible force fields proposed by Hyvönen and Kovanen [46] were considered for the systems containing