Biochimica et Biophysica Acta 1858 (2016) 872–882

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Membrane-binding properties of gating modifier and pore-blocking toxins: Membrane interaction is not a prerequisite for modification of channel gating

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ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 21 January 2016
Accepted 1 February 2016
Available online 2 February 2016

Keywords:
Venom peptide
Toxin
Gating modifier
Pore blocker
Voltage-gated ion channel
Lipid binding
Phospholipid membrane
Surface plasmon resonance
Molecular dynamics simulations

ABSTRACT

Many venom peptides are potent and selective inhibitors of voltage-gated ion channels, including channels that are validated therapeutic targets for treatment of a wide range of human diseases. However, the development of novel venom-peptide-based therapeutics requires an understanding of their mechanism of action. In the case of voltage-gated ion channels, venom peptides act either as pore blockers that bind to the extracellular side of the channel pore or gating modifiers that bind to one or more of the membrane-embedded voltage sensor domains. In the case of gating modifiers, it has been debated whether the peptide must partition into the membrane to reach its binding site. In this study, we used surface plasmon resonance, fluorescence spectroscopy and molecular dynamics to directly compare the lipid-binding properties of two gating modifiers (μ-TRTX-Hd1a and ProTx-I) and two pore blockers (ShK and KIIIA). Only ProTx-I was found to bind to model membranes. Our results provide further evidence that the ability to insert into the lipid bilayer is not a requirement to be a gating modifier. In addition, we characterised the surface of ProTx-I that mediates its interaction with neutral and anionic phospholipid membranes and show that it preferentially interacts with anionic lipids.

1. Introduction

Voltage-gated ion channels (VGICs) play crucial roles in diverse physiological processes [1,2] and are drug targets for a range of diseases, including chronic pain, multiple sclerosis, epilepsy, and cardiac arrhythmia [3–5]. Venoms from arachnids, sea anemones, cone snails, and other venomous animals are a rich source of pharmacologically active peptides [6–9] that target VGICs, especially voltage-gated sodium (Na_V), potassium (K_V), and calcium (Ca_V) channels [1,10–16]. These peptides are often highly potent and selective and have thus attracted much interest as potential lead molecules for pharmaceutical development [17–20]. However, full exploitation of their therapeutic potential requires an understanding of their mechanism of action.

VGICs are transmembrane proteins responsible for the selective transport of ions across cell membranes in response to changes in the membrane potential. They share a common architecture consisting of a central pore domain, responsible for ion conduction, and four voltage-sensing domains (VSDs) that turn the channel on or off in response to changes in the transmembrane potential [1,21–25]. The gating cycle of VGICs comprises three distinct states: closed (resting), open (activated), and in some cases, also an inactivated state. Venom peptides interfere with the gating cycle via two distinct mechanisms. Some peptides inhibit the channel by binding to the pore domain and preventing ion conduction. These peptides are referred to as pore blockers (PBs). Alternatively, some venom peptides can bind to a VSD and alter the kinetics and gating behaviour by changing the relative stability of the closed, open or inactivate states of the channel [16,21]. Peptides acting via this mechanism are called gating modifiers (GMs). As the pore domain is solvent accessible, it is likely that the binding affinity of PBs is primarily governed by peptide–protein interactions and is independent of the lipid environment surrounding the VGIC protein.

Abbreviations: Ca_V, Voltage-gated calcium channel; GMs, Gating modifier, gating-modifying peptide; Hd1a, μ-TRTX-Hd1a; K_V, Voltage-gated potassium channel; LUV, Large unilamellar vesicle; Na_V, Voltage-gated sodium channel; PBs, Pore blocker, pore blocking peptide; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; ProTx-I, Protoxin1, β-theraphotoxin-Tp1a; VGICs, Voltage-gated ion channels; VSDs, Voltage-sensing domains; SASA, Solvent accessible surface area; SUV, Small unilamellar vesicle.

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http://dx.doi.org/10.1016/j.bbamem.2016.02.002
0005-2736/

However, this has not been studied systematically. In contrast, the VSDs are largely buried in the membrane, which potentially prevents gating modifier peptides directly accessing their binding site on the VSD. Thus, gating modification is potentially a three-component system involving the peptide, the VSD, and the surrounding lipid membrane. The role of membrane partitioning and specific peptide-lipid interactions in the mechanism of GMs remains an open question. Some studies have shown that the tarantula toxins VsTx1 [26,27], SGTx1 [28,29], hanatoxin [29,30], and ProTx-II [31,32], as well as other GMs [31], partition into phospholipid bilayers. This has led to the suggestion that GMs act via a 'membrane-access mechanism' [27]. However, other studies have shown that gating modifier peptides such as huwentoxin-IV [32] do not partition into membranes [31–34] or their binding to the VSD is independent of the ability of the peptide to insert into the lipid bilayer [33]. Furthermore, contradictory results have been reported regarding the requirement of anionic phospholipids for the ability of some gating modifier peptides to insert into lipid membranes. Lee and McKinnon reported that VsTx1 binds to membranes containing some anionic phospholipids and also to membranes composed solely of zwitterionic phospholipids [27]. In contrast, Jung et al. claimed that anionic lipids are essential for membrane partitioning of VsTx1 [26]. Similarly, Milescu et al. [29] reported that SGTx1 binds to neutral and anionic phospholipids whereas in a later study Posokhov et al. reported that membrane partitioning of SGTx1 only occurs in the presence of anionic lipids [34].

A number of studies have investigated the nature of the interaction between lipids and GMs, including the position of the peptide in the lipid bilayer [26,28–30,35–37]. Based on these studies, it has been suggested that many gating modifier peptides are localised at the water–lipid interface and that the orientation of the peptide in the membrane and/or specific lipid–peptide interactions might be important for GMs to bind to VSDs. The position of the peptides and their orientation at the water–lipid interface might result from their amphipathic character. Although there is increasing evidence that the originally proposed 'membrane-access mechanism' [27] cannot be generalised to all GMs, to date there has not been a direct comparison of the phospholipid-binding activities of PBs and GMs.

In the current study, the ability of two PBs and two GMs (Fig. 1) to bind phospholipid bilayers was investigated using surface plasmon resonance (SPR), fluorescence spectroscopy, and molecular dynamics (MD) simulations. The two PBs we investigated are (i) ShK, a 35-residue peptide isolated from the sea anemone *Stichodactyla helianthus* [14,38–41]; ShK potently blocks $K_V1.3$ and is currently in Phase IIa clinical trials for the treatment of autoimmune diseases; and (ii) KIIIA, a 16-residue μ-conotoxin isolated from the cone snail *Conus kinoshitai* which inhibits tetrodotoxin-sensitive Na_V channels [42–45]. These two peptides were compared to two GMs: (i) μ-TRTX-Hd1a (Hd1a), a 36-residue peptide isolated from the tarantula *Haplopelma doriae* that selectively inhibits $Na_V1.1$ and $Na_V1.7$ [19], and (ii) ProTx-I, a promiscuous 35-residue peptide isolated from the tarantula *Thrixopelma pruriens*

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Peptide	Sequence	Source[a]	Target[b]	Charge[c]	RT (min)[d]	Inhibition[e]
ShK	RSCIDTIPKSRCTAFGCKHSMKYRLSFCRKTCGTC	*Stichodactyla helianthus*	K_V channels	+7	18.9	Pore blocker
KIIIA	CCNCSSKWCRDHSRCC	*Conus kinoshitai*	Na_V channels	+2	15.8	Pore blocker
Hd1a	ACLGFGKSCNPSNDQCCKSSSLACSTKHKWCKYEL	*Haplopelma doriae*	Na_V channels	+3	22.0	Gating modifier
ProTx-1	ECRYWLGGCSAGQTCCKHLVCSRRHGWCVWDGTFS	*Thrixopelma pruriens*	Na_V, K_V and Ca_V channels	+2	26.5	Gating modifier

[a]Animal species expressing the toxin; [b]Channels that have so far been indentified as a target; [c]Overall charge of the peptide at pH 7.4. [d]The retention time (RT) was determined by analytical-HPLC with 2%/min gradient of solvent B (90% acetonitile, 0.1% formic acid against solvent A (0.1% formic acid), this value allows comparison of the oeverall hydrophobicity of the peptides; [e]Type of inhibitor; Black lines show the disulfide connectivity

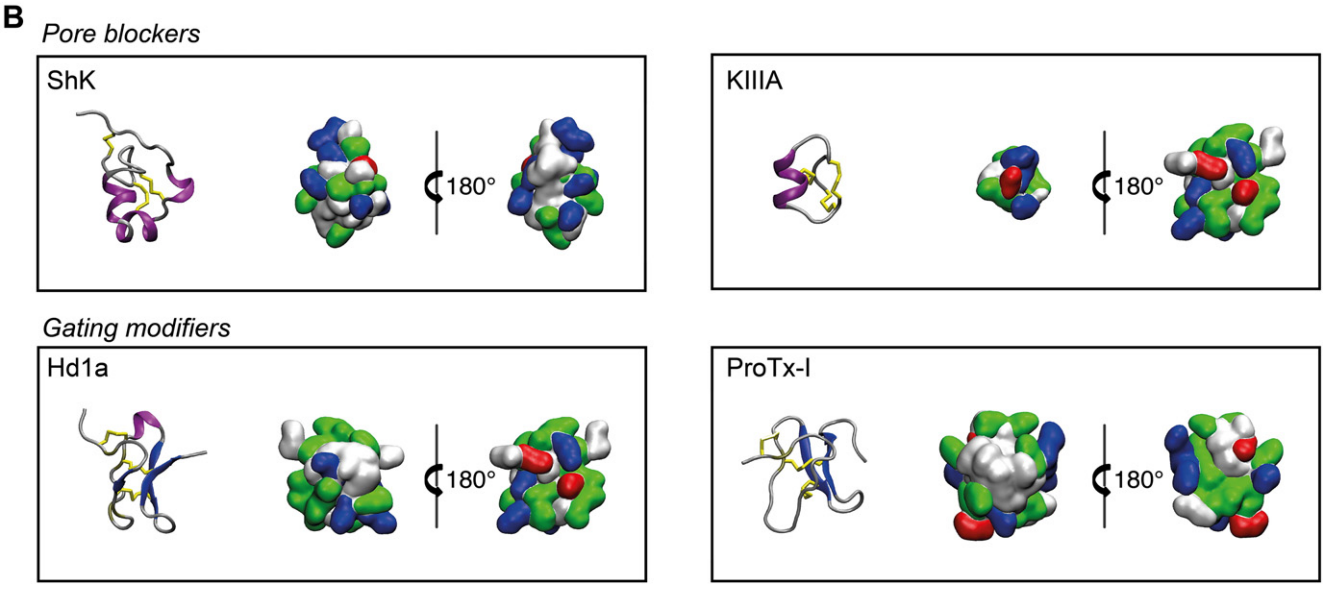

Fig. 1. *Sequence and structure of peptides used in this study.* (A) Sequence, source, ion channel target, overall charge, RP-HPLC retention time, and type of channel inhibition. Disulphide bonds are indicated by horizontal lines below the sequences. (B) Three-dimensional structure of the peptides. The ribbon representations on the left are coloured according to secondary structure (α-helices in purple and β-strands in blue), while the surface representations on the right are coloured according to residue type (hydrophobic residues in white, polar (uncharged) residues in green, positively charged residues in blue and negatively charged residues in red).