UDC 577.25 DOI: 10.25808/08697698.2018.202.6S.029

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APETx-like peptide interaction with ASICs channels: comparative *in silico* study

A comparative in silico study of the interaction of APETx-like peptide from the sea anemone Heteractis crispa with ASIC3 was carried out. According to results of protein-protein docking and MD simulations, the peptide specificity is governed by their complementarity to the pharmacophores of cASIC1 and hASIC3 but not by peptide fold.

Key words: sea anemone, APETx-like toxins, ion channels, ASIC3, molecular modeling

At present, one of the main problems of bioorganic chemistry is the investigation of molecular mechanisms of cell receptors functional activity, in particular the proton-gated ion channels (ASICs) which are expressed mainly by neurons of the central (ASIC1, ASIC2) and peripheral (ASIC3) nervous system. ASIC channels are involved in performing of the sensory neurons basic functions, such as mechanical sensitivity, perception of pain caused by inflammation and local infection. ASICs are also implicated in neurodegenerative diseases (ischemia, Parkinson's disease) [5]. However, there are relatively few ASICs modulators of peptide nature [3, 4, 6, 9, 11] compared to low molecular weight.

Previously, π-AnmTX Hcr 1b-1 (Hcr 1b-1), peptide (41 aa, 4537 Da), was obtained from the sea anemone *Heteractis crispa* by a multistage liquid chromatography. It was elicited that Hcr 1b-1 induced an inhibition of human hASIC3 peak current with an IC_{50} of 5.5 μM [11]. The BLAST search algorithm revealed that Hcr 1b1 belongs to the group of APETx2-like toxins, among which only APETx2 from the sea anemone *Anthopleura elegantissima* posseses the inhibitory effect on ASIC3 currents (IC_{50} ~ 175 and ~ 63 nM for human hASIC3 and rat rASIC3, respectively) [6]. APETx2 structure was used as a prototype for creating of Hcr 1b-1 spatial model (49% of identity) [4, 9].

The experimentally determined ASIC3 3D structure is not available yet, the channel homology model of structure was generated by program MODELLER 9.11 [8]. Currently, the spatial structures of the extracellular and transmembrane domains have been determined only for chicken cASIC1 mutants, both in the free and bound state with psalmotoxin-1 PcTx1 (IC_{50} ~ 0.9 nM for rASIC1a) from the venom of spider *Psalmopoeus cambridgei* [1, 2, 7]. The cASIC1 structure extracted from the latter complex (4FZ0) was used as a template for hASIC3 structural model generating (54% of identity). The results of molecular docking performed by ClusPro 2.0 [10] and following refinement with ToxDock servers [12] and molecular dynamics simulations of generated Hcr 1b-1–1hASIC3 complex demonstrated that hASIC3 and cASIC1 share the peptide binding sites (Fig. 1a). These sites are located between neighboring channel subunits overlapping the entrance to deep pocket with proton-binding site.

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The analysis of intermolecular interactions in Hcr 1b1–hASIC3 complex revealed the importance for binding of the peptide residues Lys5, Asp40, Arg41 (Fig. 1b) which are involved in hydrogen bonds and salt bridges formation with channel Asp163, Arg226, Asp227 ones, respectively. Interestingly, Arg226 is characteristic for ASIC3 channels, but not for ASIC1 ones with Gly at equivalent position. It should be noted that a highly basic flexible loop containing Arg26, Arg27, Arg28 residues (Fig. 1b) is responsible for the complex PcTx1–cASIC1 formation [1]. Unlike PcTx1, Hcr 1b-1 peptide is characterized by localization of basic residues His7, Lys5, and Arg41 on β-strands which leads to decreasing of both flexibility and ability to bury into deep pocket and to reach the proton-binding site (Fig. 1b). This may explain the higher IC_{50} value of Hcr 1b-1 for inhibition of hASIC3 compared to one of PcTx1 for inhibition of ASIC1. The charge inversion caused by replacement of cASIC1 Glu354Arg residue which occupies hASIC3 equal position, prevents electrostatic interactions with Arg27 of PcTx1. Moreover, in this case the important for receptor recognition by peptide πσ interactions of Arg27 with Phe174 are lost. Our molecular modeling results revealed that there are several variable residues among ASIC3 and ASIC1 at the area of peptide binding site. According to the literature data, they play a crucial role for PcTx1 binding to cASIC1 [1, 2, 7]. We suggest that Hcr 1b-1 and PcTx1 complementarity to these hASIC3 and cASIC1 variable residues is rather responsible for the binding specificity, than the difference in structure packing of peptide.

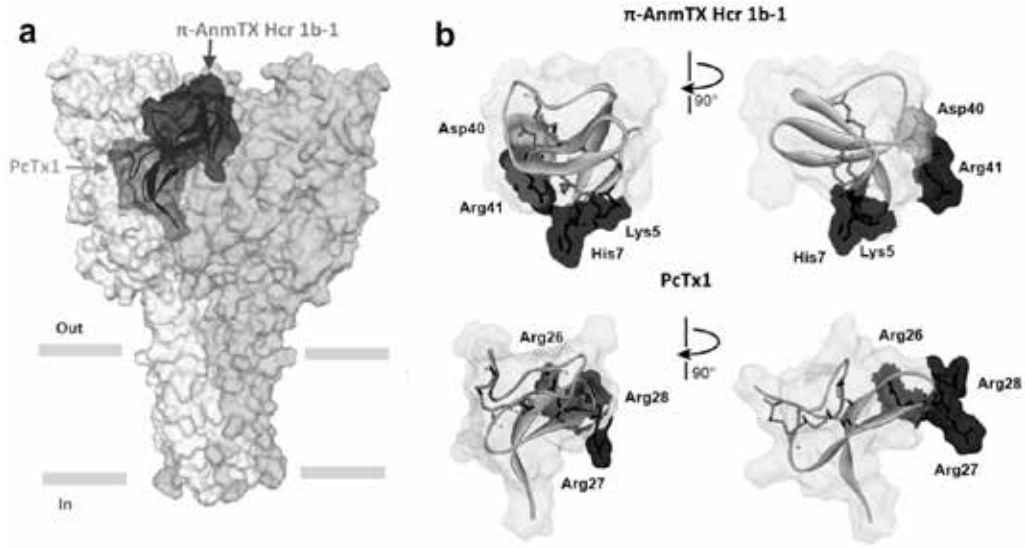

Fig. 1. The structure diagram of Hcr 1b1–hASIC3 and PcTx1–cASIC1 complexes. (a) Superposition of Hcr 1b1–hASIC3 complex structural model and X-ray structure of the PcTx1–cASIC1 (PDB ID 4FZO [1]). Each subunit of hASIC3 and cASIC1 is shown as solvent-accessible surface (grey and light-grey). Hcr 1b-1, PcTx1 are shown as ribbon diagrams (bright-grey) and transparent solvent-accessible surfaces (grey). **(b)** Hcr 1b-1 and PcTx1 [1] are shown as ribbon diagrams with transparent solvent-accessible surfaces. The disulfide bridges stabilizing peptide structures are presented as sticks. The side chains of residues important for peptides interaction with the channels, namely Lys5, His7, Arg41 (black), and Asp40 (grey) of Hcr 1b-1 and Arg26, Arg27, Arg28 (black) of PcTx1 are presented as sticks, their transparent solvent-accessible surfaces are also colored by black and grey.

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