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The combinatorial library of actinoporins from the sea anemone *Heteractis crispa*

Key words: sea anemones, actinoporins, hemolytic activity

The actinoporin family is one of four most common toxin protein families isolated from sea anemones together with sea anemone sodium channel inhibitory toxin family, type I subfamily, sea anemone type 3 potassium channel toxin family, and venom Kunitz-type family, sea anemone type 2 potassium channel toxin subfamily. It has been shown that the actinoporins are produced as isoforms; each actinoporin is encoded by its own gene.

The pore-forming mechanism has been studied in detail, but some stages of the process are still being discussed. To clarify the role of amino acid residues during membrane binding, wild-type actinoporins, as well as recombinant and mutant ones that are produced in *Escherichia coli* have been used. In general, the process of pore formation by actinoporins involves its binding to a sphingomyelin of cytoplasmic membranes through the aromatic POC site, transition of a N-terminal α-helical region (1–25 aa) to the lipid-water interface, oligomerization of 3–4, 8, or 9 monomers within the membrane interface, and the insertion of the N-terminal region into membrane hydrophobic core resulted in the creation of the functionally active protein-lipid pore. Actinoporin conformational transformation from the soluble state to the membrane-binding one is a fundamental α-PFT property that is directed at the disruption of biological targets.

Due to pore-forming activity, actinoporins represent an important model for studying of protein-membrane interactions as well as tools to investigation of action on target organs and different cell cultures and to creation of actinoporin immunoconjugates with different ligands for selective killing of parasite and tumor cells. StnII from *Stichodactyla helianthus* encapsulated into liposome have been recently reported to function as an adjuvant inducing a robust specific CTL response. Moreover, earlier we demonstrated that RTX-A from *Heteractis crispa* exhibited an antitumor effect and suppressed IGF-induced tumor transformation of JB6P + Cl41 mouse epithelial cells. This effect was found to be due to the induction of p53-independent apoptosis and the inhibition of the activity of the oncogenic nuclear factors AP-1 and NF-κB.

Several actinoporin isoforms with molecular weights of 18995.5 to 19398.7 Da exhibiting a high hemolytic activity were isolated from the tropical sea anemone *H. crispa* using a combination of liquid chromatography techniques. The sequences of the genes encoding actinoporins were identified, and the amino acid sequences of the new polypeptides belonging to the actinoporin family were obtained. The acinoporins differ in their isoelectric points, the number and localization of charged amino acid residues at the functionally important N-terminal fragment of

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the molecule, as well as in the charge of a tetrapeptide (amino acid residues 74–77) involved in an electrostatic interaction with the cytoplasmic membrane. The phylogenetic analysis revealed that actinoporin clustering is consistent with the division of sea anemones into superfamilies and families. The functional analysis of six recombinant actinoporins demonstrated that *H. crispa* actinoporin grouping was consistent with the different hemolytic activity of their representatives. According to molecular modeling data, we assume that the direction of the N-terminal dipole moment tightly reflects the actinoporins' ability to possess hemolytic activity.

The obtained data expand knowledge on the structural and functional relationships of actinoporins and contribute to our understanding of the functioning mechanism of these molecules, which is the basis for the development of compounds with a high biomedical potential.