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Review Article

The Sea Anemone Pore-Forming Toxins (PFTs): From Mechanism of Action to Perspectives in Pharmacology as Antitumor Agents

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Abstract

It is known that venomous marine coelenterates, sea anemones, widespread in the World Ocean, are producers of various biologically active compounds of protein nature: neurotoxins, Kunitz-type inhibitors, pore-forming toxins, which are the part of venom secret used by these predatory organisms for attacking and protecting against potential enemies. The study of their structure, functional activity, specificity of interaction with biological targets showed the presence of pharmacological potential in some sea anemone protein compounds. In particular, it was found that pore-forming toxins (actinoporins) possessed antitumor activity due to high cytotoxicity of these polypeptides. The results of the study of the mechanisms of actinoporins interaction with biological targets are discussed in the review. It was carried out *in vitro* (the cytoplasmic membranes of a number of tumor cells, mammalian erythrocytes, sea urchin eggs and sperm) as well as by biophysical and calculation methods (differential scanning calorimetry, computer modeling). The combination of these approaches has made it possible to significantly deepen and expand the currently existing understanding of the mechanism of an actinoporins cytolytic action. Thus, in addition to the generally accepted mechanism of an interaction between actinoporin POC-binding site and sphingomyelin, a membrane "lipid receptor" discussed in this review, an hypothesis about possible alternative binding of actinoporin RGD-motif to membrane integrin, the minimal integrin-binding motif for RGD-recognizing integrins, is put forward. Both processes determine the experimentally proven antitumor effect of actinoporins and indicate their pharmacological potential.

Keywords: *Sea Anemone; Pore-Forming Toxins (PFTs); Pharmacology; Antitumor Agents*

Introduction

To date the treatment of many diseases, including cancer, has achieved significant success due to the use of chemotherapy, which, in combination with targeted therapy, often has positive results even in the treatment of advanced stages of oncology. However, the progno-

sis is still poor for many types of cancer. Therefore, a large-scale search for the new selective antitumor compounds of natural origin as well as the production of synthetic or recombinant analogues based on them continue along with the development of new technologies for the treatment of this disease.

One of the promising producers of compounds with antitumor activity is sea anemones, venomous coelenterates living in almost all regions of the World Ocean [1,2]. These predatory animals spending their life cycle mainly in a sedentary state attached to the sea soil constitute significant danger to hydrobiologists, divers, tourists, and many predatory marine organisms as they have a unique cellular mechanism used as their own defense for injecting their venomous secret into the body of a potential victim [2]. Sea anemone venom is a real cocktail consisting of a variety of both toxic and non-toxic protein components acting on the different organs of target organisms and causes systemic poisoning, paralysis, and even death [1,3,4].

The multicomponent composition of sea anemone venom includes three main groups of protein compounds: (1) the peptide neurotoxins (3 - 5 kDa) [1,5-8], which are toxic to mammals and/or arthropods due to the ability to modify voltage-gated sodium channels (Navs) of neuro-, cardio-, skeletal muscle cells as biological targets [1,5,7-10] such as voltage-gated sodium and potassium channels (Navs and Kvs); (2) the pore-forming toxins (PFTs) or actinoporins (~20 kDa, toxic to mammals [11,12]) acting on eukaryotic cytoplasmic membranes and artificial ones and forming water-permeable pores in them [13-17] what leads to lysis and death of target cells [18]; (3) the Kunitz-type protease inhibitors, non-toxic peptides (6 kDa) involved in the degradation of proteolytic enzymes [19-23], which can also inhibit/block nociceptive TRPV1 receptor [24-26] and/or Kv channels [27].

Molecular mechanism of actinoporins action

The molecular mechanism of the actinoporin' cytolytic action (pore formation) is based on the electrostatic attraction of a positively charged molecule (Figure 1, a) to the oppositely charged eukaryotic cytoplasmic membrane with the specific interaction of a phosphorylcholine (POC) binding site on the surface of protein globule with the phosphorylcholine head groups of membrane sphingomyelin (SM) (Figure 1, b, I); this leads to "anchoring" of the molecule in the membrane interface [28]. Next, the conformational transformation of the amphiphilic N-terminal fragment (1-29 aa) occurs, affecting the SRK/KRK loop, which, like a hinge, unfolds and moves this fragment into the membrane water-lipid interface (Figure 1, b, II), where the helix is elongated by several residues and then incorporated into the membrane hydrophobic lipid core (Figure 1, b, III) [28,29]. The N-terminal fragments of 4 (or 8-9) actinoporin monomers together with membrane phospholipids form a toroidal pore (Figure 1, c) [28-31]. This mechanism of pore formation, which is characteristic of all known alpha-PFTs, underlies their cytolytic activity [18].

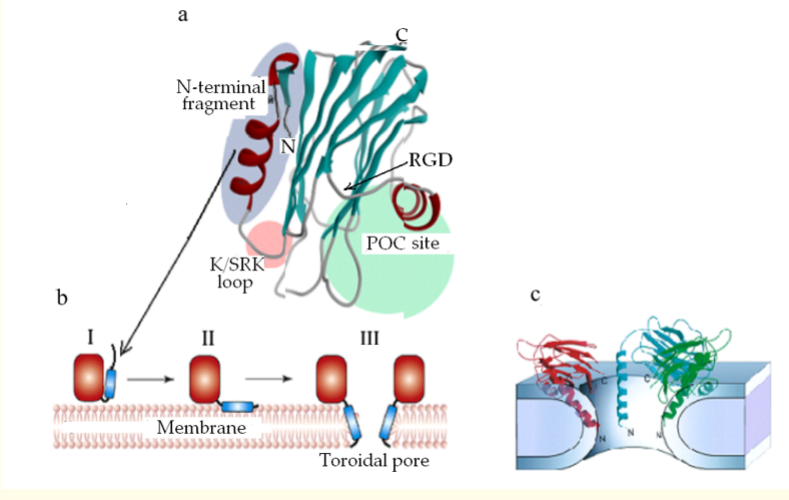

Figure 1: *(a) The ribbon diagram of the actinoporin spatial structure. (b) The mechanism of pore formation by an actinoporin. (c) The schematic representation of a pore formed by an actinoporin and a membrane [28].*

The antitumor activity

The study of the mechanism of the action of the actinoporin RTX-Ala from the sea anemone *Heteractis crispa* on cell cultures of tumors, HeLa, THP-1, MDA-MB-231, and SNU-C, as well as the epithelial cells JB6P+Cl41 showed that the actinoporin has cancer-preventive and antitumor activities, which are due to the disruption of signal transduction pathways, the induction of p53-independent apoptosis, and the inhibition of the activity of oncogenic AP-1 and NF-kB nuclear factors manifesting at the concentration of 0.0338 nM, which is 16.86 times lower than the toxic activity (0.57 nM) in relation to tumor cells [32,33]. Although the use of actinoporins as antitumor agents is currently limited by their cytotoxic effect on normal cells of a body, the actinoporin-induced lysis of tumor cells at lower concentrations than cytotoxic doses (previously found *in vivo* and *in vitro*) indicates the prospects for their research as antitumor agents.

It is noted that the content, ratio and distribution of charged and hydrophobic aliphatic and aromatic residues in the N-terminal amphiphilic fragment of the molecule (1-29 aa) is of decisive importance for actinoporin cyto- and hemolytic activity. An in silico study of the cytolytic activity of virtual peptides with sequences similar to those of N-terminal fragments (1-29 a.a.) of actinoporins from the sea anemones *Heteractis crispa*, *Stichodactyla helianthus*, *Actinia equina*, and *Actinia fragacea* showed that these peptides (Figure 2) have activity, the value of which depends on their length, hydrophobicity and hydrophobic moment, as well as the distribution of charged amino acid residues [30]). According to the calculated data, the hemolytic activity of these peptides (nmol/l) correlates as follows: EqtII>StnII>Hct-A2>RTX-S2>RTX-A>Hct-S3>StnI>Hct-A3>FraC>Hct-S6>Hct-S5>Hct-A5>Hct-A4.

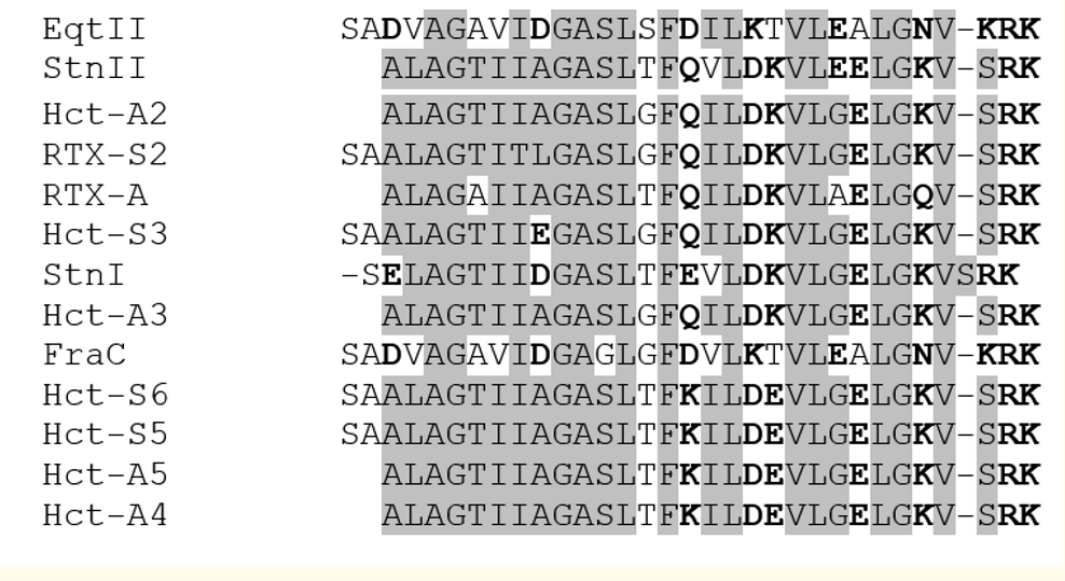

Figure 2: *The sequence alignment of peptides with sequences equal to those of the N-terminal fragments of the actinoporins from different sea anemone species: H. crispa (RTX-A,-A2,-A3,-A4,-A5; RTX-SII, Hct-S3, -S5, S6 [12]); S. helianthus (StnII and StnI [16,28]); A. equina (EqtII [13]), A. fragacea (FraC [34]). Charged amino acid residues are shown in bold, identical hydrophobic amino acid residues are shown on a light gray background.*

Computer modeling methods (alanine mutagenesis, molecular dynamics) will make it possible to determine the N-terminal sequences of actinoporins, which will have low cytolytic but high antitumor activity in the possible mutant analogues of native molecules for further obtaining pharmacologically active compounds on their basis.

The role of an actinoporin RGD motif as alternative binding site with membrane integrins

Recently we have hypothesized that, in addition to a functionally significant POC binding site and an amphiphilic N-terminal fragment involved in pore formation, the actinoporin RTX-A has an additional (or alternative) binding site to a cytoplasmic membrane, the tripeptide 141ArgGlyAsp143 (a so-called RGD motif known to be responsible for cell adhesion to the extracellular matrix). RGD tripeptide is characteristic of many membrane-active peptides and proteins, including actinoporins, and is the minimal integrin-binding motif for the RGD-recognizing integrins [35,36]. This was indicated by the previously obtained results of differential scanning calorimetry and shadow electrophoresis of human erythrocytes modified with RTX-A (Figure 3) [37]. Changes in the nature of the calorimetric melting curve of the membrane proteins of erythrocyte shadows modified with an actinoporin (the shifts of A-, B-, C-, and D-transitions of erythrocyte cytoskeleton proteins (Figure 3a and 3b) [35]) as well as actin leaching (from an erythrocyte membrane) observed in the electropherogram (Figure 3c) testified of a connection break between the cytoskeleton actin and the membrane receptor, the integrin αVβ5 [37].

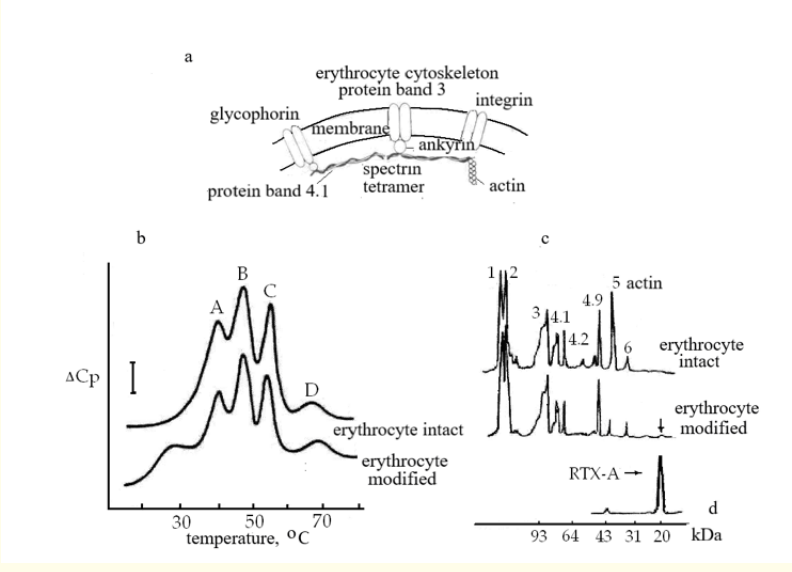

Figure 3: *(a) The schematic representation of an erythrocyte membrane fragment, membrane and cytoskeleton proteins. The calorimetric curves (b) and the densitograms of SDS electrophoresis (c) of human erythrocytes shadows intact and modified with actinoporin RTX-A [37].*

Obviously, this interaction of an RGD motif with an αVβ3 integrin may explain the ability of actinoporins to inhibit/block the processes of fertilization of the eggs of the sea urchin *Strongylocentrotus intermedius* with its sperm (on the surface of the heads of which there is an RGD tripeptide (Figure 4a and 4b)) [38]. According to the literature data, the mechanism of eggs fertilization is due to binding of the RGD tripeptide of spermatozoa to β-chain of the αVβ3 and/or αVβ5 integrins, eggs cell membrane receptors (Figure 4) [39]. Integrins are the family of transmembrane proteins, the main receptors involved in intercellular interactions [40].

The previously discovered cleavage of the bond between integrins and actin (Figure 4, b, I), the main protein of the oocyte cytoskeleton, which occurs under the action of the RGD tripeptide actinoporin and the possible leaching of actin from the oocyte membrane (similar to what occurs in erythrocytes (Figure 3c), prevents the leakage the process of eggs fertilization by sperm (Figure 4, b, II).

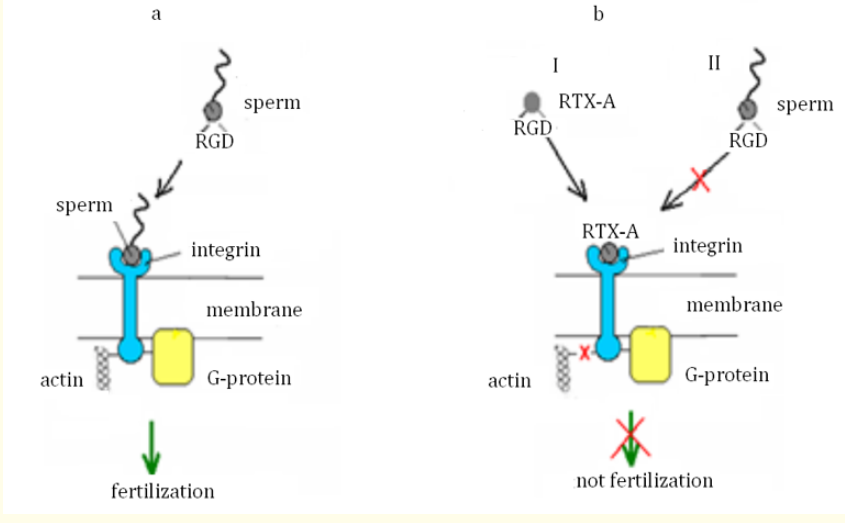

Figure 4: (a). *The scheme of the sea urchin eggs fertilization with sperm; (b) Inhibition of egg fertilization by actinoporin RTX-A.*

It should be noted that an alternative mechanism of interaction between the actinoporin RGD motif and integrin receptors in the membranes of biological targets, in particular tumor cells, can obviously be enhanced by the cytolytic action of actinoporin [18,32,33,41].

Conclusion

Thus, sea anemone α-PFT, whose structure is programmed by nature to perform a number of functions in the biocenosis (due to the presence of several functionally significant binding sites with biological targets), can be used as promising models for the creation of potential pharmacologically active substances. So literature data indicate great prospects for their use as immunotoxins, the action of which is directed to tumor cells [41].

Bibliography

1. Norton RS. "Sea anemone venom peptides". In Handbook of Biologically Active Peptides; Kastin, A.J. (Edition.) Elsevier (Academic): San Diego, CA, USA (2013): 430-436.
2. Ashwood LM., *et al*. "Tentacle morphological variation coincides with differential expression of toxins in sea anemones". *Toxins* 13 (2021): 452.
3. Macrander J., *et al*. "Tissue-specific venom composition and differential gene expression in sea anemones". *Genome Biology and Evolution* 8 (2016): 2358-2375.
4. Madio B., *et al*. "Sea anemone toxins: A structural overview". *Marine Drugs* 17 (2019): 325.
5. Moran Y., *et al*. "Sea anemone toxins affecting voltage-gated sodium channels - molecular and evolutionary features". *Toxicon* 54 (2009): 1089-1101.
6. Bosmans F and Tytgat J. "Sea anemone venom as a source of insecticidal peptides acting on voltage-gated Na+ channels". *Toxicon* 49 (2007): 550-560.

7. Liao Q., *et al*. "Cnidarian peptide neurotoxins: a new source of various ion channel modulators or blockers against central nervous systems disease". *Drug Discovery Today* 24.1 (2019): 189-197.

8. Monastyrnaya MM., *et al*. "The sea anemone neurotoxins modulating sodium channels: an insight at structure and functional activity after four decades of investigation". *Toxins* 15.1 (2023): 8.

9. Niklas B., *et al*. "Interactions of sea anemone toxins with insect sodium channel - insights from electrophysiology and molecular docking studies". *Molecules* 26 (2021): 1302.

10. Oliveira JS., *et al*. "Binding specificity of sea anemone toxins to Na(v) l.1-1.6 sodium channels-Unexpected contributions from differences in the IV/S3-S4 outer loop". *Journal of Biological Chemistry* 279 (2004): 33323-33335.

11. Valle A., *et al*. "The multigene families of actinoporins (part I): Isoforms and genetic structure". *Toxicon* 103 (2015): 176-187.

12. Leychenko E., *et al*. "Multigene family of pore-forming toxins from sea anemone Heteractis crispa". *Marine Drugs* 16 (2018): 183.

13. Anderluh G and Maček P. "Cytolytic peptide and protein toxins from sea anemones (Anthozoa: Actiniaria)". *Toxicon* 40.2 (2002): 111-124.

14. Kristan K., *et al*. "Pore formation by equinatoxin, a eukaryotic pore-forming toxin, requires a flexible N-terminal region and a stable beta-sandwich". *Journal of Biological Chemistry* 279.45 (2004): 46509-46517.

15. Bakrač B and Anderluh G. "Molecular mechanism of sphingomyelin-specific membrane binding and pore formation by actinoporins". *Advances in Experimental Medicine and Biology* 677 (2010): 106-115.

16. Alvarez C., *et al*. "The role of ionic strength on the enhancement of the hemolytic activity of sticholysin I, a cytolysin from Stichodactyla helianthus". *Toxicon* 36.1 (1998): 165-178.

17. Palacios-Ortega J., *et al*. "Evaluation of different approaches used to study membrane permeabilization by actinoporins on model lipid vesicles". *BBA - Biomembranes* 862.9 (2020): 183311.

18. Tejuca M., *et al*. "Sea anemone cytolysins as toxic components of immunotoxins". *Toxicon* 54 (2009): 1206-1214.

19. Zykova TA., *et al*. "Amino acid sequence of trypsin IV inhibitor from *Radianthus macrodactylus*". *Russian Journal of Bioorganic Chemistry* 11 (1985): 293-301.

20. Sokotun IN., *et al*. "Proteinase inhibitors from the tropical sea anemone *Radianthus macrodactylus*: Isolation and characteristic". *Russian Journal of Bioorganic Chemistry* 72 (2007): 301-306.

21. Gladkikh I., *et al*. "New HCRG-polypeptides of Kunitz-type from the sea anemone, Heteractis crispa". *Marine Drugs* 13 (2015): 6038-6063.

22. Isaeva M., *et al*. "A new multigene superfamily of Kunitz-type protease inhibitors from sea anemone Heteractis crispa". *Peptides* 34 (2012): 88-97.

23. Sintsova OV., *et al*. "Kunitz-type peptides of the sea anemone *Heteractis crispa* - potential anti-inflammatory compounds". *Russian Journal of Bioorganic Chemistry* 43.1 (2017): 91-97.

24. Andreev YA., *et al*. "Analgesic compound from sea anemone *Heteractis crispa* is the first polypeptide inhibitor of vanilloid receptor 1 (TRPV1)". *Journal of Biological Chemistry* 1022.283 (2008): 23914-23921.

25. Monastyrnaya M., *et al*. "Kunitz-type peptide HCRG21 from the sea anemone *Heteractis crispa* is a full peptide antagonist of the TRPV1 receptor". *Marine Drugs* 14 (2016): 229.

26. Peigneur S., *et al*. "A bifunctional sea anemone peptide with Kunitz type protease and potassium channel inhibiting properties". *Biochemical Pharmacology* 82 (2011): 81-90.

27. Gladkikh I., *et al*. "Kunitz-type peptides from the sea anemone *Heteractis crispa* demonstrate potassium channel blocking and anti-inflammatory activities". *Biomedicines* 8 (2020): 473.

28. Mancheno JM., *et al*. "Crystal and electron microscopy structures of Sticholysin II actinoporin reveal insights into the mechanism of membrane pore formation". *Structure* 11 (2003): 1319-1328.

29. Malovrh P., *et al*. "A Novel mechanism of pore formation: membrane penetration by the N-terminal amphipathic region of equinatoxin". *Journal of Biological Chemistry* 278 (2003): 22678-22685.

30. Ros U., *et al*. "Differences in activity of actinoporins are related with the hydrophobicity of their N-terminus". *Biochimie* 116 (2015): 70-78.

31. Tanaka K., *et al*. "Structural basis for self-assembly of a cytolytic pore lined by protein and lipid". *Nature Communications* 6 (2015): 6337.

32. Fedorov S., *et al*. "The anticancer effects of actinoporin RTX-A from the sea anemone *Heteractis crispa* (=Radianthus macrodactylus)". *Toxicon* 55 (2010): 811-817.

33. Kvetkina A., *et al*. "Sea anemone Heteractis crispa actinoporin demonstrates *in vitro* anticancer activities and prevents HT-29 colorectal cancer cell migration". *Molecules* 25 (2020): 5979.

34. Morante K., *et al*. "Functional characterization of Val60, a key residue involved in the membrane-oligomerization of fragaceatoxin C, an actinoporin from *Actinia fragacea*". *FEBS Letters* 589 (2015): 1840-1846.

35. Danhier F., *et al*. "RGD-based strategies to target alpha(v) beta(3) integrin in cancer therapy and diagnosis". *Molecular Pharmaceutics* 9.11 (2012): 2961-2973.

36. García Linares S., *et al*. "The sea anemone actinoporin (Arg-Gly-Asp) conserved motif is involved in maintaining the competent oligomerization state of these pore-forming toxins". *The FEBS Journal* 281.5 (2014): 1465-1478.

37. Shnyrov VL., *et al*. "Calorimetric study of interactions of toxin from *Radianthus macrodactylus* with erythrocyte membrane". *Biochem International* 26 (1992): 219-229.

38. Monastyrnaya MM., *et al*. "9th IST Asia Pacific meet". on Animal, Plant and Microbial Toxins, Vladivostok, Russia 4-8 (2011): 53.

39. Burke RD., *et al*. "Integrin signaling in early sea urchin development". *Signal Transduction* 7 (2007): 207-215.

40. Murray G., *et al*. "The αBβC Integrin is expressed on the surface of the sea urchin egg and removed at fertilization". *Developmental Biology* 227 (2000): 633-647.

41. Rivera-de-Torre E., *et al*. "Pore-forming proteins from cnidarians and arachnids as potential biotechnological tools". *Toxins* 11 (2019): 370.

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