

Prospects of sea anemone peptides for pharmacology

Abstract

It has been found that the genes of a number of protein components of sea anemone venoms underwent significant diversification in the course of evolution. The elucidation of the molecular mechanisms of sea anemone peptides interactions with targets let investigators in the last decade actively study the molecular organization and the functioning mechanisms of cytoplasmic membranes, the various types and subtypes of ion channels/receptors involved in the processes of perception, processing, intra- and intercellular signal transduction, both in a body physiological and pathological state.

Keywords: *molecular organization, sea anemone, protein toxins, arthropods*

Introduction

The relevance of the research

The search for natural compounds with pharmacological potential, the study of their diversity consistent patterns of evolutionary development, structure-functional relationships with biological targets, and an effective application in biotechnology and medicine are priority research areas in various fields of life sciences. At the same time, researchers face one of the most urgent tasks. It is the creation of agents highly specific to various targets and having high therapeutic potential aimed at treating socially significant diseases (cancer, neurodegenerative, cardiovascular, etc.) [1]. It is known that marine coelenterates (sea anemones, jellyfishes) producing a huge variety of protein toxins are the most attractive and studied producers of biologically active substances (along with sea snakes and cone mollusks, as well as terrestrial venomous organisms: snakes, arthropods, insects) used for creation promising drugs for pharmacology on their basis [2].

It has been found that the genes of a number of protein components of sea anemone venoms underwent significant diversification in the course of evolution. Due to functionalization, sub- and neofunctionalization, they formed multigene families encoding combinatorial peptide libraries: APETx-like toxins [3-5], pore-forming toxins (actinoporins) [6], Kunitz-type peptides [7], which were evolutionarily selected highly homologous molecules with functional activity aimed at expanding of biological targets in the body of a prey or a potential predator. The search for new natural peptide ligands with high specificity to different biological targets is an urgent task that researchers face [1,2].

The analysis of the current state of research in this area

To date it has been established that the major components of sea anemone venomous secrets are: neurotoxins (5 kDa) (Figure, **a**, **b**) [8], some toxic and non-toxic APETx-like peptides (4 kDa) (Figure, **c**) [3-5], pore-forming toxins (PFTs) (20 kDa) (Figure, **d**) [6,9], as well as non-toxic Kunitz-type peptides (6 kDa) (Figure, **e**) [7,10-12] and β -defensin-like peptides (4 kDa) (Figure, **f**) [13]. Site-directed mutagenesis, electrophysiological and in silico studies have shown that the selective action of sea anemone protein ligands and their activity are conditioned by the interaction of functionally significant residues with their binding sites on the appropriate biological targets: voltage-gated Nav [8] and Kv [14] ion channels (for **a** and **b**), acid-sensing (ASICs) channels [3-5] (for **e**) and many other ones [15], cytoplasmic membranes (for **d**) [16],

proteases [7,10-12] and/or TRPV1 [17,18] and H-1 histamine [19] receptors (for **e**), pancreatic alpha-amylase [13,20] (for **f**). Using modern biotechnological omics technologies and approaches (genomics, transcriptomics, proteomics as well mass spectrometry), high-throughput NGS and in vitro screening (cell biology and electrophysiology) made it possible to study the structural diversity and functional specificity of toxins produced by sea anemones, as well as the molecular mechanisms of their interaction with various targets [21].

The elucidation of the molecular mechanisms of sea anemone peptides interactions with targets let investigators in the last decade actively study the molecular organization and the functioning mechanisms of cytoplasmic membranes, the various types and subtypes of ion channels/receptors involved in the processes of perception, processing, intra- and intercellular signal transduction, both in a body physiological and pathological state [2]. Thus, it has been reliably established that violation of the functional activity of targets causes various types of channelopathies and pathologies while the blocking or activating effect of sea anemone peptides can have a pharmacological effect on them. So toxic and non-toxic sea anemone venomous peptides, due to their unique and individual properties as well as their possessing large structural diversity and having selectivity/specificity for various targets, are of great interest as a basis for the design of new potential therapeutic agents.

At the moment, a very small amount of effective and safe protein ligands is observed to act selectively on certain ion channels and exhibit a therapeutic effect [22]. From the three structural types of toxins modulating Kv channels, the bifunctional toxins of type 2, kalicludines AsKC 1 – AsKC 3 (*Actinia viridis*, 58–59 aa) are the most studied, they inhibit both trypsin and Kv1.2 and calcium (Ca^{2+})-dependent potassium channels [23]. The presence of pharmacological action was reliably shown only for type 1 toxin, ShK from *Stichodactyla helianthus* (35 amino acid residues) and its recombinant analog, 20kDa-PEG-[Lys16]ShK [23]. Both of them bind selectively to Kv1.1 and Kv1.3 of T-lymphocytes without affecting other cell types, are involved in the regulation of membrane potential and signal transduction, and inhibit the secretion of interleukins IL-17 and IL-4 in monkeys [24]. This ability may be promising in the treatment of autoimmune diseases (multiple sclerosis, rheumatoid arthritis).

Among the fifty representatives of the four structural types (1–4) of neurotoxins (identified to date) (Figure, **a**, **b**) modulating Nav channels and slowing down the kinetics of their rapid inactivation upon binding to site 3, no pharmacological effect has been found [8]. At the same time, highly homologous sea anemone APETx-like peptides with a similar structural fold, non-toxic peptides Hcr 1b-1 – Hcr 1b-4 (*Heteractis crispa*) (Figure, **c**) are able to inhibit acid-sensing ASIC1a and ASIC3 channels, exhibiting an anxiolytic effect and sufficiently high anti-inflammatory activity [4,5]. It has been established that, in addition to ASIC3, APETx2 (*Anthopleura elegantissima*) (Figure, **c**) inhibits ASIC1a/3, ASIC1b/3, and ASIC2b/3 channels, effectively potentiates ASIC1b and ASIC2a ones [3], showing an analgesic effect in the models of acid-induced muscle pain as well as one caused by inflammation.

It has been found that actinoporins StnI from *S. helianthus* and RTX-A (=Hct-A) from *H. crispa* (Figure, **d**), have antitumor activity [16]. Thus, Hct-A is cytotoxic to colon cancer cells (SNU-C4) [21], monocytic leukemia (THP-1), cervical cancer (HeLa), and breast cancer (MDA-MB-231). It prevents epidermal growth factor-induced tumor transformation of mouse JB6P+Cl41 epithelial cells by activating p53-independent apoptosis and inhibiting the activity of oncogenic nuclear factors AP-1 and NF- κ B [25]. In recent years the actinoporins StnI, gigantoxin-4 (*Stichodactyla gigantea*), EqtII (*Actinia equina*), and FraC (*Actinia fragacea*) have been used for creating immunoconjugates with various ligands for targeted cytolytic action on parasitic and tumor cells [26].

The multigene family of *H. crispa* Kunitz-type peptides [7] (Figure, **e**) is a source of a huge variety of representatives which activity determined by certain functionally significant residues is also associated with acting on various targets [10,11,15]. Among the representatives of this family, some belonging to so-called “analgesic cluster” [7] have also been found: APHC1–APHC3 [7,17], HCRG21 [18], HCRG1, HCRG2, HCGS1.10, HCGS1.36, HCGS1.19,

HCGS1.20 [7, 10-12] (Figure, e), which activity is due to both a trypsin-inhibiting effect [19,20] and inhibition of a vanilloid (TRPV1) receptor [17,18] and some Kvs [15]. As a result, the peptides exhibit pronounced analgesic and anti-inflammatory activity.

Thus, it is clear that the quantitative increase of the targets of pharmacologically active sea anemone peptides improves prospects for successful development of new venom-based therapeutics.

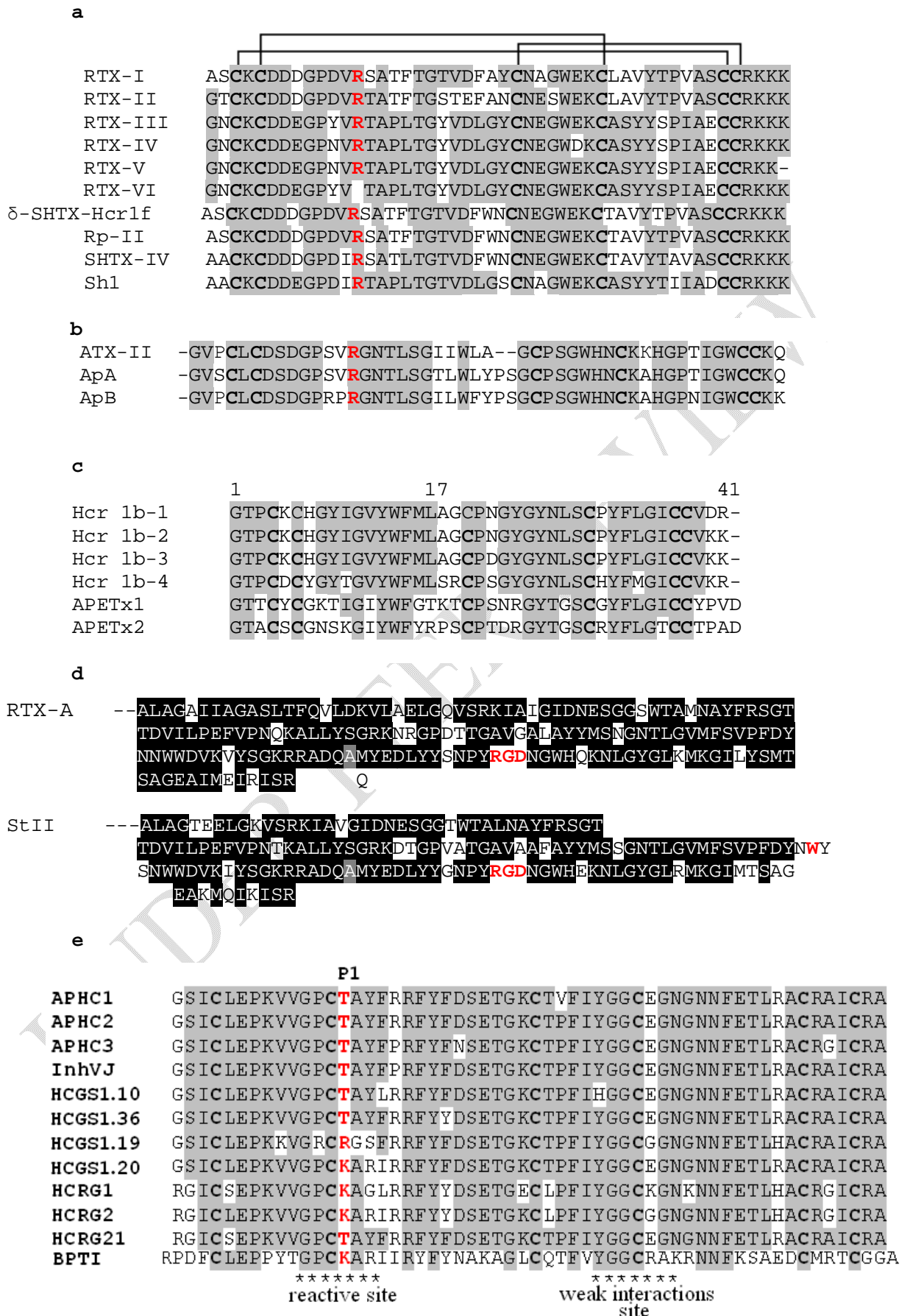
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UNDER PEER REVIEW



f

Magnificamide	SEGT SCYIYHGVYGI CKAKCAEDMKAMAGMGV CEGDLCCYKTPW
Helianthamide	ESGN SCYIYHGV SG ICKASCAE DEKAMAGMGV CEGHLCCYKTPW

Figure.1. Multiple sequence alignments of representatives of sea anemone peptides and polypeptides belonging to the different peptide groups, such as neurotoxins of type 2 (**a**): RTX-I, RTX-II, RTX-III, RTX-IV, RTX-V, RTX-VI, δ -SHTX-Hcr1f from *Heteractis crispera*, Rp-II from *Radianthus paumotensis*, SHTX-IV from *Stichodactyla haddoni*, Sh1 from *Stichodactyla helianthus*; neurotoxins of type 1 (**b**): ATX-II from *Anemonia sulcata*, ApA, ApB from *Anthopleura xanthogrammica* [8]; APETx-like peptides (**c**): Hcr 1b-1 – Hcr 1b-4 [4,5] from *H. crispera* and APETx1, APETx2 from *A. elegantissima* [3]; pore-forming toxins (**d**): RTX-A from *H. crispera* [6] and StII from *S. helianthus* [9]; Kunitz-type peptides (**e**): APHC1 – APHC3 [7], InhVJ [10], HCRG1, HCRG2 [7,11], , HCGS1.10, HCGS1.36, HCGS1.19, HCGS1.20 from *H. crispera* [7,12], HCRG21 [7]; β -defensin-like peptides (**f**): magnificamide from *H. magnifica* and helianthamide from *S. helianthus* [13]. Highly homologous residues and/or sequence fragments are indicated in color. Identical amino acid residues are shown on a gray (**a, b, c**) and black (**d**) background, point substitutions of residues in the sequences are shown on white; Arg13 residue, functionally significant for the binding of neurotoxins to Na_vs (**a, b**), as well as RGD tripeptide binding of PFTs to integrins (**d**) are highlighted in red. The P1 residue is shown (**e**): residues Lys, Arg, Thr, which determine the interaction of Kunitz peptides with targets, proteases and/or TRPV1 receptor, are highlighted in red. The straight lines at the top of (**a**) show C1-C5, C2-C4, C3-C6 disulfide bridges between cysteine residues (shown in bold). All alignments are made with the help program Vector NTI.