Peptide Toxins in Sea Anemones: Structural and Functional Aspects

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Abstract

Sea anemones are a rich source of two classes of peptide toxins, sodium channel toxins and potassium channel toxins, which have been or will be useful tools for studying the structure and function of specific ion channels. Most of the known sodium channel toxins delay channel inactivation by binding to the receptor site 3 and most of the known potassium channel toxins selectively inhibit Kv1 channels. The following peptide toxins are functionally unique among the known sodium or potassium channel toxins: APETx2, which inhibits acid-sensing ion channels in sensory neurons; BDS-I and II, which show selectivity for Kv3.4 channels and APETx1, which inhibits human ether-a-go-go-related gene potassium channels. In addition, structurally novel peptide toxins, such as an epidermal growth factor (EGF)-like toxin (gigantoxin I), have also been isolated from some sea anemones although their functions remain to be clarified.

Keywords: peptide toxin — potassium channel toxin — sea anemone — sodium channel toxin

Introduction

Members of the phylum Cnidaria commonly possess specialized stinging organelles (nematocysts) to capture prey animals. On chemical or physical stimulation, the thread tubule folded in the nematocyst is discharged and penetrates the epithelium of the victim. Simultaneously, toxins in the nematocyst enter the victim through the thread tubule, leading to its paralysis. Apart from the inherent biological function in sea anemones, nematocyst toxins from some species of sea anemones such as Anemonia sulcata and Phyllodiscus semoni are even dangerous to humans. When an individual is stung by nematocysts, local inflammations, including severe pain, redness, and edema are immediately induced by toxins.

In general, sea anemone toxins are considerably stable compared to other cnidarian toxins (typically jellyfish toxins). Thus, a number of toxins have so far been isolated from various species of sea anemones and well characterized, although it is not always clear whether these toxins are derived from nematocysts. Most of the sea anemone toxins are divided into the following three classes: 20-kDa pore-forming cytolysins inhibitable by sphingomyelin [now called actinoporins; Kem, 1988; Anderluh and Macek, 2002], 3- to 5-kDa neurotoxins acting on voltage-gated sodium channels [Kem, 1988; Kem et al., 1990; Norton, 1991], and 3.5- to 6.5-kDa neurotoxins acting on voltage-gated Kv1 potassium channels [Castañeda et al., 1995; Schweitz et al., 1995; Cotton et al., 1997; Gendeh et al., 1997; Minagawa et al., 1998a]. Of the three classes of toxins, both sodium and potassium channel peptide toxins have been useful tools for studying the structure and function of ion channels, because of their high affinity to the specific channel. Besides the well-characterized peptide toxins, structurally and/or functionally novel peptide toxins, which seem to be promising pharmacological reagents, have recently emerged from some species of sea anemones.

In this article, accumulated knowledge on the structural and functional aspects of sea anemone peptide toxins is reviewed, with special emphasis on sea anemones as an important source of fascinating pharmacological tools. The three-dimensional structure–function relationships have been clarified for some sea anemone peptide toxins (Gasparini et al., 2004; Mouhat et al., 2004) but are not included in this review because of space limitations.

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Sodium Channel Peptide Toxins

Since the first discovery of three toxins in *Anemonia sulcata* [Béress et al., 1975], more than 50 sodium channel peptide toxins have been isolated and/or cloned from various species of sea anemones. As proposed by Norton (1991), most of the sea anemone sodium channel toxins can be classified into three types based on the determined amino acid sequences [Figure 1]. As listed in Table 1, as many as 33 and 9 toxins have been identified as type 1 and 2 toxins, respectively. For type 1 and 2 toxins, therefore, only some typical sequences are included in Figure 1.

Type 1 and 2 toxins are composed of 46 to 49 amino acid residues, except for Ae I of 54 residues [Lin et al., 1996], and cross-linked by three disulfide bridges (4–46, 6–36, and 29–47; numbering is based on the amino acid sequence of ApA). Ten residues including six Cys residues are completely conserved between type 1 and 2 toxins. In view of the fact that a toxin (halcurin) with structural features of both type 1 and 2 toxins is present in *Halcurias* sp. [Ishida et al., 1997a], the most primitive species belonging to the suborder Endocoelantheae compared to the other species so far studied of the suborder Nynantheae, both type 1 and 2 toxins are considered to have evolved from the same ancestor gene. However, they are immunologically distinguishable from each other because no antigenic cross-reactivity between both types of toxins is recognized [Schweitz et al., 1985; Kem et al., 1989]. It is interesting to note that the distribution of type 1 and 2 toxins seems to be related to the taxonomical position of sea anemones; members of the family Actiniidae contain only type 1 toxins, while either type 1 or 2 toxins or both type 1 and 2 toxins are found in those of the family Stichodactylidae.

**Table 1**

| Type 1     | 1 | 10 | 20 | 30 | 40 |
|------------|---|----|----|----|----|
| ApA        | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| ApB        | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| ATX II     | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| Ae I       | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| Cp I       | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| Rc I       | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |
| AFT I      | GVCCLDSGPDSVGNLTSGLLWYPS | GCPGWNCNHAPGTIGWCK |

**Type 1 + Type 2**

| Halcurin   | 1 | 10 | 20 | 30 | 40 |
|------------|---|----|----|----|----|
| VACRCE5GDPDVRSAFTGTVDLW | NCNTGWKCIAYTAVASCCKD |

**Type 2**

| RTX I      | 1 | 10 | 20 | 30 | 40 |
|------------|---|----|----|----|----|
| GNCDDGDPVDSATFTGVDA | YCNAGWKEKCLAYTPVASCRRK |
| Rp III     | 1 | 10 | 20 | 30 | 40 |
| GNCDDGDPVDSATFTGVDA | YCNAGWKEKCLAYTPVASCRRK |
| Sh I       | 1 | 10 | 20 | 30 | 40 |
| GNCDDGDPVDSATFTGVDA | YCNAGWKEKCLAYTPVASCRRK |
| Gigantoxin III | 1 | 10 | 20 | 30 | 40 |
| GNCDDGDPVDSATFTGVDA | YCNAGWKEKCLAYTPVASCRRK |

**Type 3**

| PaTX       | 1 | 10 | 20 | 30 | 40 |
|------------|---|----|----|----|----|
| ACGKSTCCPCACWCKYTAGCPWQQ | CAAHGCCE |
| Da I       | 1 | 10 | 20 | 30 | 40 |
| ACGKSTCCPCACWCKYTAGCPWQQ | CAAHGCCE |
| Da II      | 1 | 10 | 20 | 30 | 40 |
| ACGKSTCCPCACWCKYTAGCPWQQ | CAAHGCCE |
| Er I       | 1 | 10 | 20 | 30 | 40 |
| ACGKSTCCPCACWCKYTAGCPWQQ | CAAHGCCE |
| ATX III    | 1 | 10 | 20 | 30 | 40 |
| RCCPCMVGGCPVWQQ | YCPSGPKV |

**Others**

| Calitoxin I | 1 | 10 | 20 | 30 | 40 |
|-------------|---|----|----|----|----|
| GCAPDLSHM+GTG+VFSC+KGG+DSWSK+CN+YT+A+ADC+CHQA |

**Fig. 1.** Amino acid sequences of sodium channel toxins from sea anemones. ApA and ApB are from *Anthopleura xanthogrammica* [Tanaka et al., 1977; Reimer et al., 1985]; ATX II from *Anemonia sulcata* [Wunderer et al., 1976]; Ae I from *Actinia equina* [Lin et al., 1996]; Cp I from *Condylactis passiflora* [Shiomi et al., 1995]; Rc I from *Radianthus (Heteractis) crispus* [Sunahara et al., 1987]; halcurin from *Halcurias* sp. [Ishida et al., 1997a]; RTX I and RTX II from *Radianthus (Heteractis) macrodactylus* [Zykova et al., 1988a, b]; Rp III from *Radianthus (Heteractis) paumotensis* [Mettrione et al., 1987]; Sh I from *Stichodactyla helianthus* [Kem et al., 1989]; gigantoxin III from *Stichodactyla gigantea* [Shiomi et al., 2003]; PaTX from *Entacmaea Parasicyonis* actinostoloides [Nishida et al., 1985]; Da I and Da II from *Dofleinia armata* [Honma et al., 2003a]; Er I from *Entacmaea raamsayi* [Honma et al., 2003a]; ATX III from *Anemonia sulcata* [Martinez et al., 1977]; calitoxins I and II from *Calliactis parasitica* [Cariello et al., 1989, Spagnuolo et al., 1994]. Hydroxy-Pro at position 3 of Cp I and Rc I are denoted by “O.” Identical amino acid residues with ApA, RTX I, PaTX, and calitoxin I are boxed for type 1, 2, and 3 toxins and calitoxins, respectively. Asterisks represent the common amino acid residues for both type 1 and 2 toxins. The lines above and below the sequence of halcurin indicate the residues peculiar to type 1 and 2 toxins, respectively.
Type 3 sodium channel toxins are shorter peptides composed of 27 to 32 amino acid residues. Previously, two toxins, PaTX from *Entacmaea actinostoloides* (formerly called *Parasicyonis actinostoloides*; Nishida et al., 1985) and ATX III from *Anemonia sulcata* (Martinez et al., 1977), have been tentatively classified into type 3 toxins (Norton, 1991). However, PaTX and ATX III are cross-linked by four and three disulfide bridges, respectively, implying that they share no structural scaffold. In our recent study, two toxins (Da I and II) isolated from *Dofleinia armata* and one toxin (Er I) from *Entacmaea ramsayi* were found to be homologous with PaTX (Honma et al., 2003a), suggesting a wide distribution of PaTX-like toxins in sea anemones. Therefore, it may be reasonable to include only PaTX and its analogs in the category of type 3 toxins.

At least six different receptor sites for neurotoxins are known for the mammalian sodium channels. Similar to α-scorpion toxins, sea anemone type 1–3 toxins bind to the receptor site 3 of sodium channels and prolong the open state of the channels during the depolarization procedure (Catterall and Bércess, 1978; Vincent et al., 1980; Schweitz et al., 1981; Warashina et al., 1988a,b; Norton, 1991). Because of this unique action on the sodium channels, some of the known sea anemone sodium channel toxins, such as ATX II from *Anemonia sulcata* (Wunderer et al., 1988) and anthopleurin A (ApA; Tanaka et al., 1977) and B (ApB; Reimer et al., 1985) from *Anthopleura xanthogrammica*, have been used as valuable pharmacological reagents in many laboratories of the world. Detailed molecular studies on the interaction with sodium channels have been performed using ApB and its various site-directed mutants. The results show that the flexibility of the region 8–17 (Arg-14 loop) is essential for toxin binding to sodium channels (Seibert et al., 2003). As for individual residues, Arg-12 within the Arg-14 loop and Leu-18 and Ser-19 proximal to the C-terminus of the loop greatly contribute to toxin affinity (Gallagher and Blumenthal, 1994; Dias-Kadambi et al., 1996; Seibert et al., 2004). It is worth mentioning that ApB has no selectivity for neuronal and cardiac sodium channels, while ApA is selective for cardiac channels. This difference in selectivity between ApA and ApB is associated with

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**Table 1. Distribution of Type 1 and 2 Sodium Channel Toxins in Sea Anemones**

| Species                        | Type 1 toxins | Type 2 toxins | References                          |
|--------------------------------|---------------|---------------|-------------------------------------|
| **Family Actiniidae**          |               |               |                                     |
| *Actinia equina*               | Ae I          |               | Lin et al., 1996                    |
| *Anemonia erythrae*            | AETX I        |               | Shiomi et al., 1997                 |
| *Anemonia sulcata*             | ATX Ia and Ib |               | Widmer et al., 1988                 |
|                                | ATX II        |               | Wunderer et al., 1976               |
|                                | ATX V         |               | Scheffler et al., 1982              |
| **Anthopleura elegansissima**  | ApC           |               | Norton, 1981                        |
|                                | APE 1–1, 1–2, and 2–2 |               | Bruhn et al., 2001                  |
| **Anthopleura fuscoviridis**   | AFT I and II  |               | Sunahara et al., 1987               |
| **Anthopleura xanthogrammica** | ApA           |               | Tanaka et al., 1977                 |
|                                | ApB           |               | Reimer et al., 1985                 |
|                                | PCR1–2, 2–1, 2–5, 2–10, 3–6, and 3–7 |               | Kelso and Blumenthal, 1998          |
| **Anthopleura sp.**            | Hk2a, 7a, 8a, and 16a |               | Wang et al., 2004                   |
| **Bunodosoma caissarum**       | Be III        |               | Malpezzii et al., 1993              |
| **Bunodosoma canicium**        |               |               | Cunha et al., 2005                  |
| **Bunodosoma granulifera**     | Bg II and III |               | Loret et al., 1994                  |
| **Condylactis passiflora**     | Cp I and II   |               | Shiomi et al., 1995                 |
| **Family Stichodactylidae**    |               |               |                                     |
| **Antheopsis maculata**        | Am III        |               | Honma et al., 2005                  |
| **Radianthus (Heteractis) crispus** | Rc I          |               | Shiomi et al., 1996                 |
| **Radianthus (Heteractis) macrodactylus** | RTX I   | RTX II         | Zykova and Kozlovskaya, 1989        |
|                                |               | RTX III        | Zykova et al., 1988a                |
|                                |               | RTX IV and V   | Zykova et al., 1988b                |
|                                |               | Rp II          | Schweitz et al., 1985               |
|                                |               | Rp III         | Metrione et al., 1987               |
| **Radianthus (Heteractis) paumotensis** | Gigantoxin II | Gigantoxin III | Shiomi et al., 2003                 |
| **Stichodactyla gigantea**     |               |               |                                     |
| **Stichodactyla helianthus**   |               |               |                                     |

Type 3 sodium channel toxins are shorter peptides composed of 27 to 32 amino acid residues. Previously, two toxins, PaTX from *Entacmaea actinostoloides* (formerly called *Parasicyonis actinostoloides*; Nishida et al., 1985) and ATX III from *Anemonia sulcata* (Martinez et al., 1977), have been tentatively classified into type 3 toxins (Norton, 1991). However, PaTX and ATX III are cross-linked by four and three disulfide bridges, respectively, implying that they share no structural scaffold. In our recent study, two toxins (Da I and II) isolated from *Dofleinia armata* and one toxin (Er I) from *Entacmaea ramsayi* were found to be homologous with PaTX (Honma et al., 2003a), suggesting a wide distribution of PaTX-like toxins in sea anemones. Therefore, it may be reasonable to include only PaTX and its analogs in the category of type 3 toxins.
the replacements at positions 12 and 49 [Khera et al., 1995]. Furthermore, experiments using mutants of either neuronal sodium channels [Rogers et al., 1996] or cardiac sodium channels [Benzinger et al., 1998] suggested that the receptor site 3 is involved in the extracellular loop IVS3-S4 and that anionic residues (Glu-1613 and Glu-1616 for neuronal channels and Asp-1612 for cardiac channels) in the IVS3-S4 loop electrostatically interact with basic residues of toxins. However, more recent study using six different sodium channel genes (Nav1.1-1.6) showed that some nearby amino acids, which are different in the channels, contribute to the interaction with toxins [Oliveira et al., 2004].

Besides the type 1–3 toxins described above, two novel sodium channel toxins, calitoxins I and II (46 amino acid residues) distinguishable by only one replacement at position 6, have been isolated or cloned from Calliactis parasitica [Cariello et al., 1989; Spagnuolo et al., 1994]. Both calitoxins are comparable to type 1 and 2 toxins as to chain length and disulfide bridge pattern but their entire amino acid sequences greatly differ from those of type 1 and 2 toxins. They act on voltage-gated sodium channels probably in a similar manner to type 1–3 toxins.

**Potassium Channel Peptide Toxins**

It has not been long since sea anemone potassium channel peptide toxins were discovered in the mid-1990s. Nevertheless, much knowledge on their structures and functions has been accumulated in the last decade. Based on the structural and functional differences, the 11 potassium channel peptide toxins so far isolated can be classified into three types as shown in Figure 2.

Type 1 potassium channel toxins blocking Kv1 (Shaker) potassium channels include ShK from Stichodactyla helianthus [Castañeda et al., 1995], AsKS [kaliseptine] from Anemonia sulcata [Schweitz et al., 1995], BgK from Bunodosoma granulifera [Cotton et al., 1997], HmK from Heteractis magnifica [Gendeh et al., 1997], and AeK from Actinia equina [Minagawa et al., 1998a]. These toxins are composed of 35 to 37 amino acid residues and cross-linked by three disulfide bridges (3–35, 12–28, and 17–32; numbering is based on the amino acid sequence of ShK). Alanine scanning experiments identified three residues, Ser-20, Lys-22, and Tyr-23, as essential for the binding of ShK to rat brain potassium channels [Pennington et al., 1996]. Similar experiments carried out on BgK also proved that the corresponding residues (Ser-23, Lys-25, and Tyr-26) are involved in the binding to rat brain potassium channels [Dauplais et al., 1997] and Kv1.1, Kv1.2, Kv1.3, and Kv1.6 channels [Alessandri-Haber et al., 1999; Gilquin et al., 2002]. These three residues are completely conserved in the other type 1 toxins. Of the three residues, the dyad (Lys-Tyr) is considered to be especially important for the binding to potassium channels. It is interesting to note that scorpion toxins blocking Kv1 channels, such as charybdotoxin and margatoxin, contain a similar dyad composed of Lys and a hydrophobic residue (e.g., Lys-27 and Tyr-36 for charybdotoxin), which is critical for their binding to Kv1 channels [Dauplais et al., 1997; Gasparini et al., 2004].

**Fig. 2.** Amino acid sequences of potassium channel toxins from sea anemones. ShK is from Stichodactyla helianthus [Castañeda et al., 1995]; AsKS [kaliseptine]; AsKS 1-3 [kalicludines 1–3]; BDS-I and BDS-II from Anemonia sulcata [Schweitz et al., 1995; Diochot et al., 1998]; BgK from Bunodosoma granulifera [Cotton et al., 1997]; HmK from Heteractis magnifica [Gendeh et al., 1997]; AeK from Actinia equina [Minagawa et al., 1998a]; APETx1 from Anthopleura elegantissima [Diochot et al., 2003]. Identical residues with ShK, AsKC 1, and BDS-I are boxed for type 1, 2, and 3 toxins, respectively. For reference, the amino acid sequences of dendrotoxin I, a potassium channel toxin from black mamba, and BPTI [bovine pancreatic trypsin inhibitor] are aligned with those of type 2 toxins.
Although there is a distinct difference in molecular scaffold between sea anemone type 1 potassium channel toxins and scorpion potassium channel toxins, the important dyads of both toxins superimpose in the three-dimensional structures. It is thus assumed that structurally unrelated toxins [sea anemone and scorpion toxins] have undergone convergent evolution to bind to the same region of structurally related targets (Gasparini et al., 2004).

Type 2 potassium channel toxins, AsKC 1-3 (kalicludines 1–3), are composed of 58 or 59 amino acid residues and also exhibit blocking of Kv1 channels with much less potency than type 1 toxins (Schweitz et al., 1995). In accordance with the sequence homologies with Kunitz-type protease inhibitors, such as bovine pancreatic trypsin inhibitor (BPTI), AsKCs have protease inhibitory activity although less potently than BPTI. As compared to the three residues [Lys-15, Ala-16, and Ile-19] of BPTI, which are known to be important to bind to trypsin, AsKCs have a significant replacement (Ile by Pro) at position 19, leading to their weaker inhibitory activity than BPTI. In relation to this, dendrotoxins, Kv1 channel blockers from either green or black mamba, also share high sequence homologies with Kunitz-type protease inhibitors but exhibit no protease inhibitory activity.

It should be noted that various species of sea anemones contain Kunitz-type protease inhibitors (Wunderer et al., 1981; Zykova et al., 1985a; Antuch et al., 1993; Ishida et al., 1997b; Minagawa et al., 1997, 1998b). Sea anemone protease inhibitors have been considered to function to inhibit endogenous proteases in animals themselves or to protect the toxins injected into prey animals or predators from rapid degradation. However, the finding of potassium channel toxins with protease inhibitory activity, such as AsKCs, leads us to assume that sea anemone protease inhibitors serve not only as defensive substances but also as offensive substances to paralyze prey animals. It is interesting to examine whether the sea anemone protease inhibitors so far isolated have potassium channel toxicity.

Three toxins, BDS-I and II from Anemonia sulcata (Diochot et al., 1998) and APETx1 from Anthopleura elegantissima (Diochot et al., 2003), are members of type 3 potassium channel toxins. BDS-I and II are the first specific blockers of Kv3.4 channels and are substantially inactive to other members of the Kv3 (Shaw) subfamily. Although they show about 25% sequence identities with type 1 sodium channel toxins such as ApA and ATX II, they elicit no effect on sodium channels in cardiac and skeletal muscle cells; they have only a weak effect on tetrodotoxin-sensitive sodium channels in neuroblasts. On the other hand, APETx1 has 64%, 42%, and 42% sequence identities with APETx2, BDS-I, and BDS-II, respectively, and also shares the same structural scaffold with them but its target channel is different. It is a selective blocker of human ether-a-go-go-related gene (HERG or erg1) potassium channels. Besides APETx1, two scorpion toxins, ErgTx from Centruroides noxius (Gurrola et al., 1999) and BeKm-1 from Buthus eupeus (Korolkova et al., 2001), have also been reported to be potent inhibitors of HERG channels.

**Acid-Sensing Ion Channel Peptide Toxin**

APETx2 (42 amino acid residues) isolated from Anthopleura elegantissima (Diochot et al., 2004) is functionally quite unique. Although it shares 36% to 64% sequence identities with type 3 potassium channel toxins, BDS-I and II from Anemonia sulcata and APETx1 from Anthopleura elegantissima (Figure 3), it inhibits not potassium channels but acid-sensing ion channels (ASICs, H+-gated sodium channels; Waldmann and Lazdunski, 1998) in sensory neurons, which are implicated in the modulation of pain sensation. ASICs are formed by homomeric or heteromeric association of six different subunits [ASIC1a, ASIC1b, ASIC2a, ASIC2b,
ASIC3, and ASIC4) and only ASIC3 channels and ASIC3-containing channels are affected by APETx2. Until the discovery of APETx2, psalmotoxin 1 (PcTX1) from the tarantula Psalmopoeus cambridgei has been the sole toxin acting on ASICs (Escoubas et al., 2000). However, APETx2 has no sequence homology with PcTX1 and is functionally discriminated from PcTX1 inhibiting homomeric ASIC1a channels. Since APETx2 is the first specific inhibitor for ASIC3 channels and ASIC3-containing channels, it will be a promising tool to study the physiological involvement of ASIC3 channels in neuronal excitability and pain coding.

Other Structurally Novel Peptide Toxins

In addition to the peptide toxins described above, the following structurally novel peptide toxins have been isolated although not functionally characterized: AETX II and III from Anemonia erythraea (Shiomi et al., 1997), gigantoxin I from Stichodactyla gigantea (Shiomi et al., 2003; Honma et al., 2003b), and Am I and II from Antheopsis maculata (Honma et al., 2005a).

AETX II and III are composed of 59 amino acid residues and are highly homologous with each other (Figure 4). There are only four substitutions between both toxins. These toxins are featured by the presence of as many as 10 Cys residues probably forming five disulfide bridges. Moreover, they are most potently lethal to crabs among the peptide toxins so far isolated from sea anemones by our laboratory; the LD50 values of AETX II and III against crabs are 0.53 and 0.28 μg/kg, respectively.

Gigantoxin I, which is not lethal but potently paralytic to crabs with an ED50 of 215 μg/kg, was discovered through a careful observation of the symptoms of crabs injected with samples. Very surprisingly, it shows about 35% sequence homologies with epidermal growth factors (EGFs) from mammals (Figure 5). Consistent with the structural resemblance in Figure 5, gigantoxin I displays EGF activity, as evidenced by rounding of human epidermoid carcinoma A431 cells and tyrosine phosphorylation of the EGF receptor in the cells, although much less potently than human EGF. The finding of an EGF-like molecule (gigantoxin I) with both toxic and EGF activities in the sea anemone S. gigantea, which is the nearest to the phylogenetic root of the animal kingdom, allows us to assume that the ancestors of EGFs originally had functioned as toxins as in the case of gigantoxin I and that they had lost toxic properties during the evolution process.

Am I (27 amino acid residues) appears to act on sodium channels from the lethality to crabs (LD50 830 μg/kg). Differing from all the known sea anemone peptide toxins, Am I has only four Cys residues, suggesting its unique conformation to be clarified (Figure 6). It is also interesting to note that the Am I precursor contains as many as six copies of Am I. On the other hand, Am II (46 amino acid residues) is only paralytic to crabs (ED50 420 μg/kg), similar to gigantoxin I. It should be emphasized that the crab assay is a simple and useful tool to discover new toxins, such as Am II and gigantoxin I, if only the symptoms induced in crabs by samples are carefully observed. It is worth mentioning that Am
II is homologous to APETx2 (ASIC3 channel blocker), BDS-I (Kv3.4 channel blocker), BDS-II (Kv3.4 channel blocker), and APETx1 (HERG channel blocker) with 28%, 39%, 39%, and 37% sequence identities, respectively (Figure 6). Despite the structural similarity, APETx2, BDS-I, BDS-II, and APETx1 target different ion channels. Am II may act to specialized ion channels or one of the ion channels targeted by these four toxins.

The sea anemone peptide toxins described above are all derived from the whole bodies, tentacles or secreted mucus. However, we recently found that the extract from special aggressive organs (acrorhagi) of *Actinia equina* is toxic to crabs. The acrorhagi are located in a ring around the base of the tentacles in certain species of sea anemones belonging to the family Actiniidae and used to fight with nonspecific non-clonemates. Two novel peptide toxins, acrorhagins I (50 amino acid residues) and II (44 amino acid residues), were isolated from the acrorhagi of *A. equina* (Figure 7; Honma et al., 2005b). Acrorhagin I has no sequence homologies with any toxins from other biological sources. On the other hand, acrorhagin II is somewhat homologous (20% to 27% identity) with hainantoxin-I (sodium channel toxin) from the Chinese bird spider *Selenocosmia hainana* [Li et al., 2003], α-conotoxin MVIB (calcium channel toxin) from the cone snail *Conus magus* [Olivera et al., 1985], and Tx 3-2 from the Brazilian armed spider *Phoneutria nigriventer* [Cordeiro et al., 1993], are aligned with that of acrorhagin II. Identical residues with acrorhagin II are boxed.

![Fig. 7. Amino acid sequences of acrorhagins I and II from acrorhagi of *Actinia equina* (Honma et al., 2005b). The amino acid sequences of three peptide toxins, hainantoxin-I from the Chinese bird spider *Selenocosmia hainana* [Li et al., 2003], α-conotoxin MVIB from the cone snail *Conus magus* [Olivera et al., 1985], and Tx 3-2 from the Brazilian armed spider *Phoneutria nigriventer* [Cordeiro et al., 1993], are aligned with that of acrorhagin II. Identical residues with acrorhagin II are boxed.](image)

Concluding Remarks

In the early 1990s, it was once concluded that sodium channel toxins binding to the receptor site 3 are the sole family of sea anemone peptide toxins. However, potassium channel peptide toxins have emerged as a new family of peptide toxins in the mid-1990s and structurally and functionally novel peptide toxins have also been discovered one after another. As a result of extensive studies on sea anemone peptide toxins, some of them have been used as valuable tools in studying the structure and function of ion channels. Importantly, only about 40 species have been examined for peptide toxins, although more than 800 species of sea anemones are recorded in the world. In the course of our screening for toxins in sea anemones, all species tested have been found to contain toxins that are lethal or paralytic to crabs, suggesting the universal distribution of peptide toxins in sea anemones. This article ends with the hope that future study on sea anemone peptide toxins will discover fascinating new molecules acting on specific ion channels, expanding our understanding of the structure and function of various ion channels deeply implicated in the physiology of animals.

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