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New view on crotamine, a small basic polypeptide myotoxin from South American rattlesnake venom

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Abstract

Crotamine is a toxin from the *Crotalus durissus terrificus* venom, composed of 42 amino acid residues and three disulfide bridges. It belongs to a toxin family previously called Small Basic Polypeptide Myotoxins (SBPM) whose members are widely distributed through the *Crotalus* snake venoms. Comparison of SBPM amino acid sequences shows high similarities. Crotamine induces skeletal muscle spasms, leading to spastic paralysis of the hind limbs of mice, by interacting with sodium channels on muscle cells. The crotamine gene with 1.8 kbp is organized into three exons, which are separated by a long phase-1 and short phase-2 introns and mapped to chromosome 2. The three-dimensional structure of crotamine was recently solved and shares a structural topology with other three disulfide bond-containing peptide similar to human β-defensins and scorpion Na^+ channel toxin. Novel biological activities have been reported, such as the capacity to penetrate undifferentiated cells, to localize in the nucleus, and to serve as a marker of actively proliferating living cells.

Keywords: Crotalus; Myotoxin; Crotamine; Cationic peptide; Structure; Cell penetrating peptide; Biological activity; Gene; Paralogous gene; Toxin

Contents

1. Introduction

Crotamine was first observed in the venom of Argentinean rattlesnakes (*Crotalus durissus terrificus*) by

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Gonçalves and Polson (1947). Later on, this extremely basic toxin and inductor of spastic paralysis of the hind limbs of mice was found in other rattlesnake venoms from the southern region of Brazil (Gonçalves and Vieira, 1950). Gonçalves (1956) described an individual crotamine polymorphism in venoms after which Schenberg (1959) related this characteristic to geographic rattlesnake distribution. It was only at the end of the 70′s, that several toxins with similar chemical and biological properties were isolated from the venom of other rattlesnake species: myotoxin *a* from *C. viridis viridis* (Cameron and Tu, 1977; Fox et al., 1979), peptide C from *C. v. helleri* (Maeda et al., 1978), myotoxin I and II from *C. v. concolor* (Bieber et al., 1987), CAM toxin from *C. adamanteus* (Samejima et al., 1991), crotamine-Ile 19 from *C. d. ruruima* (Santos et al., 1993) and E toxin from *C. horridus horridus* (Allen et al., 1996). There is a high degree of amino acid conservation among these members, with scores varying from 83 to 98%. Ownby (1998) designated these toxins Small Basic Polypeptide Myotoxins (SBPM). Fig. 1 shows the amino acid sequences alignment and the evolutionary relationship of these toxins.

General knowledge concerning the SBPM is based on myotoxin *a* (Bieber and Nedelkov, 1997) and crotamine research. Interestingly, despite the fact that SBPM has been considered a homogeneous group due to the conserved amino acid sequences, research on toxin interactions with muscle cells was conducted by emphasizing distinct receptors. The works with myotoxin *a* were driven to Ca^{2+} influx via calsequestrin or Ca^{2+} ATPase (Bieber and Nedelkov, 1997), whereas the approach used to investigate crotamine interaction was with Na^{+} channels (Chang and Tseng, 1978; Pellegrini Filho et al., 1978; Matavel et al., 1998). In structural protein analysis, the solution structure of crotamine was resolved by Siqueira et al. (2002), and Nicastro et al. (2003). In molecular genetics, the crotamine gene was isolated, its structural organization determined and its chromosomal location mapped by Rádis-Baptista et al. (2003). Later, in the scope of cell biology, Kerkis et al. (2004) revealed new biological activities of crotamine by using mouse embryonic stem cells. In this work our objective is to present recent progress on crotamine research.

2. Crotamine polymorphism

Brazilian rattlesnake venoms present several levels of divergence in their composition, such as geographic variation (Schenberg, 1959), variation of crotamine content (Gonçalves and Arantes, 1956; Oguiura et al., 2000) and variation of crotamine sequence (Laure, 1975; Smith and Schmidt, 1990; Santos et al., 1993; Rádis-Baptista et al., 1999; Toyama et al., 2000).

Fig. 1 shows the evolutionary relationship of SBPM. We observed that primary sequences from *C. d. terrificus*, described by Smith and Schmidt (MYX 4_CRODU and MYX1_CRODU), are not in the same phylogenetic branch as crotamine (CXRSMT) and crotamine-Ile19 (MYX_CRODR), from *C. d. terrificus* and *C. d. ruruima*, respectively. Equivalent divergence was observed between myotoxin I and II, which were purified from pooled venom samples from *C. v. concolor* (MYX1_CROVC and MYX2_CROVC). This divergence might indicate that new isoforms of SBPM are emerging or have already evolved in the course of genetic diversification.

3. Crotamine structure

Crotamine is composed of 42 amino acids (Laure, 1975) and contains, for the most part, β-sheet, α-helix and random coil structures (Kawano et al., 1982; Beltran et al., 1985, 1990). Initially, some attempts were unsuccessful in solving the crotamine 3D structure based on protein crystallization and X-ray diffraction. Some of the difficulties observed in attaining crystals may be due to several crotamine isoforms, different conformations adopted by the toxin (Hampe, 1978; Endo et al., 1989) and the formation of aggregates (Beltran et al., 1985; Teno et al., 1990). However, Siqueira et al. (2002) proposed a theoretical 3D model for crotamine using computational calculation that was accomplished by homology modeling procedure and by intensive molecular dynamics simulations. They identified a fold common to β-defensin and antopleurine-B. Finally, with a practical approach of NMR spectroscopy, Nicastro et al. (2003) solved the solution structure of crotamine. The structure comprises a short N-terminal α-helix and a small antiparallel triple-stranded β-sheet arranged in a $\alpha\beta_1\beta_2\beta_3$ topology never before encountered among active toxins on ion channels. Interestingly, crotamine not only has disulfide bridges arrangement identical to members of the human β-defensin family, but also has similar structural fold conformation (Fig. 2).

4. Biological activity

Crotamine induces spastic paralysis in the hind limbs of mice, rats, rabbits and dogs (Gonçalves, 1956). Furthermore, pharmacological studies showed that myotoxin induces depolarization of membrane potential in skeletal muscle cells and influx of Na^{+}, and this effect is prevented by tetrodotoxin as well as by decrease of extracellular concentration Na^{+} (Chang et al., 1983). This suggests that crotamine acts on the Na^{+} channel of plasmatic membrane of skeletal muscle, thus inducing the increase of this cation (Pellegrini Filho et al., 1978; Tsai et al., 1981). The alterations of Na^{+} current were measured by loose patch clamp technique in frog skeletal muscle (Matavel et al., 1998). Other activities ascribed to crotamine are increase of the basal release of acetylcholine and dopamine in rat