Toxicon 55 (2010) 558–569

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Biological and proteomic analysis of venom from the Puerto Rican Racer (*Alsophis portoricensis*: Dipsadidae)

Caroline L. Weldon, Stephen P. Mackessy*

School of Biological Sciences, University of Northern Colorado, 501 20th Street, CB 92, Greeley, CO 80639-0017, USA

ARTICLE INFO

Article history:
Received 12 August 2009
Received in revised form
27 September 2009
Accepted 2 October 2009
Available online 14 October 2009

Keywords:
Biological roles
Colubrid
CRISP
Enzymes
α-Fibrinogenase
Gland histology
Hemorrhage
Mass spectrometry
Metalloproteinase
Toxin

ABSTRACT

The Puerto Rican Racer *Alsophis portoricensis* is known to use venom to subdue lizard prey, and extensive damage to specific lizard body tissues has been well documented. The toxicity and biochemistry of the venom, however, has not been explored extensively. We employed biological assays and proteomic techniques to characterize venom from *A. portoricensis anegadae* collected from Guana Island, British Virgin Islands. High metalloproteinase and gelatinase, as well as low acetylcholinesterase and phosphodiesterase activities were detected, and the venom hydrolyzed the α-subunit of human fibrinogen very rapidly. SDS-PAGE analysis of venoms revealed up to 22 protein bands, with masses of ~5–160 kDa; very little variation among individual snakes or within one snake between venom extractions was observed. Most bands were approximately 25–62 kD, but MALDI-TOF analysis of crude venom indicated considerable complexity in the 1.5–13 kD mass range, including low intensity peaks in the 6.2–8.8 kD mass range (potential three-finger toxins). MALDI-TOF/TOF MS analysis of tryptic peptides confirmed that a 25 kDa band was a venom cysteine-rich secretory protein (CRiSP) with sequence homology with tigrin, a CRiSP from the natricine colubrid *Rhabdophis tigrinus*. The venom was quite toxic to NSA mice (*Mus musculus*: $LD_{50} = 2.1$ μg/g), as well as to *Anolis* lizards (*A. carolinensis*: 3.8 μg/g). Histology of the venom gland showed distinctive differences from the supralabial salivary glands (serous vs. mucosecretory), and like the Brown Treesnake (*Boiga irregularis*), another rear-fanged snake, serous secretory cells are arranged in densely packed secretory tubules, with little venom present in tubule lumina. These results clearly demonstrate that venom from *A. portoricensis* shares components with venoms of front-fanged snakes as well as with other rear-fanged species. Venom from *A. portoricensis*, in particular the prominent metalloproteinase activity, likely serves an important trophic function by facilitating prey handling and predigestion of prey.

1. Introduction

A prominent evolutionary trend seen among the advanced snakes is toward the production of toxic oral secretions (venoms) and away from constriction as the dominant method of subduing prey (Kochva, 1987). Venoms vary in complexity and composition, in part as a function of phylogeny and diet, as well as the influence of several other factors (Mackessy, 2009). An important biological role of venom in snakes is the immobilization of potentially dangerous, struggling prey, and by injecting venom which slows, paralyzes or kills the prey, the snake secures a meal and avoids injury. Venom of many species, particularly viperids, also produces extensive tissue necrosis and likely acts to increase rates of digestion of large prey items by "predigesting" the prey (Thomas and Pough, 1979; Mackessy, 1988). Because many venomous snakes are capable of consuming prey items of much larger

* Corresponding author. Tel.: +1 970 351 2429; fax: +1 970 351 2335.
E-mail address: stephen.mackessy@unco.edu (S.P. Mackessy).

0041-0101/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2009.10.010

size in proportion to their bodies than non-venomous snakes (Greene, 1997), the increased surface area available for gastric digestion caused by the venom breaking down the prey internally as the snake digests the prey's external surface is selectively advantageous (Mackessy, 1988).

The paraphyletic family "Colubridae" is the largest family of modern snakes, containing at least 700 venomous species world-wide (Cadle, 1994; Vidal, 2002). This clade of advanced snakes has been the subject of numerous morphological and molecular analyses, and the phylogenetic hypotheses of recent work (Vidal et al., 2007; Quijada-Mascareñas and Wüster, 2009) will be followed here; however, the term "colubrid" will be used generically and for convenience. These snakes vary greatly in size, distribution and diet, and although various species are not closely related, many possess enlarged rear teeth and a Duvernoy's gland (Weinstein and Kardong, 1994), a homolog of the venom gland of front-fanged snakes. Colubrid snakes are generally considered harmless, but there have been documented human fatalities from envenomation by the Boomslang (*Dispholidus typus*) (Pope, 1958) and species from three other genera of colubrids (*Thelotornis, Rhabdophis* and *Philodryas*) (FitzSimons and Smith, 1958; Mittleman and Goris, 1976; Kornalik and Taborska, 1978; Ogawa and Sawai, 1986; Prado-Franceschi et al., 1996). It is probable that the "non-venomous" nature of most colubrids is not due to a lack of enzymes or toxins in the venom, but instead is a result of evolution of venoms that differentially affect non-mammalian taxa (Mackessy et al., 2006; Pawlak et al., 2009). With the vast majority of colubrid venoms still unstudied, there is opportunity to discover novel biological compounds with possible medical implications; however, one obstacle to the characterization of colubrid venoms is the exceedingly small quantities of raw material previously obtainable (Mackessy, 2002). Most colubrids are of a small size and, therefore, yield small amounts of venom. While this previously hindered study, efficient extraction is now possible (Hill and Mackessy, 1997, 2000).

Snake venoms are of considerable interest pharmacologically because they contain compounds useful as probes of ion channels and other physiological processes and as leads for drug development. Colubrid snakes typically have less complex venoms than those of front-fanged snakes (Viperidae, Elapidae), but they share many of the same components including metalloproteinases, serine proteases, phosphodiesterases, acetylcholinesterases, phospholipases A_2 (PLA_2) and three-finger toxins (3FTXs) (Mackessy, 1998, 2002, 2009; Hill and Mackessy, 2000; Fry et al., 2003; Pawlak et al., 2006). Several of these (metalloproteinase and acetylcholinesterase activities) have been previously reported in the venom of the Puerto Rican Racer, *Alsophis portoricensis* (Hegeman, 1961).

Metalloproteinases are the most abundant and diverse enzymatic proteins found in most viperid venoms (Fox and Serrano, 2005; Pahari et al., 2007). Hemorrhagic metalloproteinases are responsible for the severe local inflammation and tissue necrosis seen in human envenomations (Rucavado et al., 1995, 1999), and are biologically very important to the predigestion effects of these venoms on prey (Mackessy, 1988, 1993b). Colubrid envenomations are commonly characterized by minor to significant bleeding (Kuch and Mebs, 2002) due in large part to the presence of hemorrhagic toxins (Takeya and Iwanaga, 1998), which can be serine proteinases or metalloproteinases, although hemorrhage is not necessarily due only to the proteinase components. Metalloproteinases may also show muscle-damaging activity, and myotoxic snake venom metalloproteinases have been isolated from the venoms of several colubrid snakes (e.g., *Philodryas olfersii*: Prado-Franceschi et al., 1998; *Rhabdophis tigrinus*: Komori et al., 2006; *P. patagoniensis*: Peichoto et al., 2007).

The Puerto Rican racer (*Alsophis portoricensis*), now considered a member of the family Dipsadidae (Vidal et al., 2007), is a rear-fanged colubrid snake found on numerous islands in the Caribbean. *Alsophis portoricensis* are ground-dwelling, diurnal snakes that have been observed envenomating prey before consumption (Rodriguez-Robles and Thomas, 1992). The diet of *A. portoricensis* primarily includes lizards (*Anolis* sp.) and *Eleutherodactylus* frogs, but they have also been observed preying on dead fish, bird eggs, iguanas, other snakes, and rats (Rodriguez-Robles and Leal, 1993a, b; Norton, 1993). Although typically hesitant to bite when confronted, *A. portoricensis* has been documented to cause cases of human envenomation resulting in edema, paraesthesia and ecchymosis at the bite site (Heatwole and Banuchi, 1966; R. Platenberg and K. Lindsay, pers. comm.). Because venom composition appears to be related to prey type and form (Mackessy, 1988; Daltry et al., 1996; Gibbs and Mackessy, 2009), the types of toxins utilized by particular snake species to facilitate prey handling may differ depending on the type of prey animal utilized. For example, venom from the Brown Treesnake (*Boiga irregularis*) has been shown to possess taxon-specific toxins that affect birds and reptiles to a much greater extent than mammals, and this snakes feeds primarily on non-mammalian prey (Mackessy et al., 2006; Pawlak et al., 2009). *Alsophis portoricensis* venom may also be more toxic to reptiles than to mammals because reptiles make up the majority of the diet of this snake. Hegeman (1961) commented on the hemolytic and proteolytic activities of *A. portoricensis* venom, but was unable to characterize it further due to a lack of both crude material and technical sophistication. The predatory use of venom by *A. portoricensis* has been documented, and envenomated *Anolis* lizards appeared to suffer respiratory distress resulting from hemorrhage in the lungs (Prieto-Hernandez, 1985), perhaps due to the action of SVMPs. In the current study, biological assays and proteomic techniques were employed to characterize more fully the venom of *A. portoricensis* and to provide a biochemical explanation for the effects of envenomation previously described. We also hypothesized that like *B. irregularis* venom, *A. portoricensis* venom would have taxon-specific effects, with greater potency toward lizard prey.

2. Materials and methods

2.1. Reagents

Protein concentration reagents were purchased from BioRad Inc. (San Diego, CA). Novex Mark 12 unstained molecular mass standards, MES running buffer, LDS sample