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Biochemistry and toxicology of toxins purified from the venom of the snake *Bothrops asper*

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ABSTRACT

The isolation and study of individual snake venom components paves the way for a deeper understanding of the pathophysiology of envenomings – thus potentially contributing to improved therapeutic modalities in the clinical setting – and also opens possibilities for the discovery of novel toxins that might be useful as tools for dissecting cellular and molecular processes of biomedical importance. This review provides a summary of the different toxins that have been isolated and characterized from the venom of *Bothrops asper*, the snake species causing the majority of human envenomings in Central America. This venom contains proteins belonging to at least eight families: metalloproteinase, serine proteinase, C-type lectin-like, L-amino acid oxidase, disintegrin, DC-fragment, cystein-rich secretory protein, and phospholipase A_2. Some 25 venom proteins within these families have been isolated and characterized. Their main biochemical properties and toxic actions are described, including, in some cases, their possible relationships to the pathologic effects induced by *B. asper* venom.

1. Introduction

The harmful and even fatal consequences of snakebites have been noted by mankind since the times of ancient civilizations. Venomous snakes can inspire both fear and fascination, and have been linked to mystical and religious concepts in many cultures throughout history. It is not surprising that with the advent of modern science, snake venoms became the subject of intense studies aimed at understanding their biochemical composition, and the modes by which they cause harmful effects.

Snake venoms are toxic secretions produced by a pair of specialized exocrine glands connected to the fangs by ducts (Kochva et al., 1980; Mackessy and Baxter, 2006). Such secretions are complex mixtures of molecules of different biochemical nature, with a predominance of proteins, many of which are endowed with enzymatic activities (Jiménez-Porras, 1970; Tu, 1977). This heterogeneous nature of venom composition was evidenced since the earliest analytical studies, and hence associated with the wide variety of bioactivities, both in vitro and in vivo, that were observed clinically or experimentally. Thus, it became widely established that specific activities of a snake venom could be attributed to particular components or toxins. While this general principle has been very useful and important in the study of venoms, it is not always valid, as there may be effects that are caused by two or more toxins acting in combination, i.e. synergistically. Moreover, a given toxin may have more than one specific activity, and therefore, it may play multiple roles in the overall effects of envenoming. Notwithstanding these considerations, the isolation and characterization of individual venom components constitutes the mainstay of toxinology, as a key strategy to dissect and to analyze the complex series of events involved in envenomings. Thus, steered by the development and refinement of chromatographic techniques, early studies of snake venoms using whole, unfractionated secretions rapidly evolved into detailed

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analyses of their individual components. The purpose of this review is to provide an updated summary of the different toxins that have been isolated and characterized from the venom of *Bothrops asper*, the snake species causing the majority of human envenomings in Central America (Gutiérrez, 1995).

2. Toxins isolated from *B. asper* venom

The first biochemical analyses of *B. asper* venom attempting to dissect its constituents and to establish their correlation with different enzymatic activities are probably those of Jiménez-Porras (1964), who utilized starch gel electrophoresis to separate at least 14 fractions from the venom of this species (at the time classified as *Bothrops atrox*). Differences in the venom composition of *B. asper* from the Pacific and the Caribbean (Atlantic) regions of Costa Rica were noticed since early studies on its biochemical and toxicological properties (Jiménez-Porras, 1964; Gutiérrez et al., 1980; Aragón and Gubenšek, 1981). Variations in the frequency of occurrence of some electrophoretic bands, as well as in the activity levels of several enzymes and pharmacological effects were described. For example, *B. asper* specimens inhabiting the Caribbean versant of Costa Rica were shown to produce venom that is more hemorrhagic and myotoxic than that of specimens from the Pacific versant, which display higher proteolytic activity (Gutiérrez et al., 1980).

Proteomic analyses have now revealed the presence of proteins belonging to at least eight families in *B. asper* venom: metalloproteinase (41–44%), phospholipase A_2 (29–45%), serine proteinase (4–18%), L-amino acid oxidase (5–9%), disintegrin (1–2%), C-type lectin-like (0.5%), cysteine-rich secretory protein (CRISP) (0.1%), and DC-fragment (<0.1%) (Alape-Girón et al., 2008). Representative toxins of some of these protein families have been isolated, as listed in Table 1. A few of them have been characterized biochemically and structurally, and have been the subject of a number of studies aimed at understanding their mechanisms of action. Other toxins have only been described with partial biochemical and functional characterizations. The following sections summarize the main properties and actions of the toxins listed in Table 1, and in cases where such information has been obtained, their relationships to the pathologic effects induced by *B. asper* venom.

Table 1
Proteins isolated from the venom of the snake *Bothrops asper*.

Protein family	Toxin name	Properties	Sequence, PDB[a]	Reference
Phospholipase A_2				
D49	PLA I	acidic, 32 kDa	–	Ferlan and Gubenšek (1978)
	PLA II	acidic, 16 kDa	–	Ferlan and Gubenšek (1978)
	PLA_2 1	acidic, 11 kDa	–	Alagón et al. (1980)
	PLA_2 2	acidic, 11 kDa	–	Alagón et al. (1980)
	PLA_2 3	acidic, 29 kDa	–	Alagón et al. (1980)
	myotoxin I	basic, 15 kDa	–	Gutiérrez et al. (1984)
	myotoxic PLA_2	basic, 15 kDa	–	Mebs and Samejima (1986)
	myotoxin III	basic, p*I* 8.7, 15 kDa	P20474	Kaiser et al. (1990)
K49	myotoxin II	basic, p*I* 9.1, 15 kDa	P24605, 1CLP	Lomonte and Gutiérrez (1989)
	myotoxin IV	basic, 15 kDa	–	Díaz et al. (1995)
Metalloproteinase				
P-I	proteinase G	neutral, p*I* 7.1, 18 kDa	–	Aragón and Gubenšek (1987)
	BaP1	basic, p*I* 8.2, 23 kDa	P83512, 1ND1	Gutiérrez et al. (1995a)
P-III	BaH1	acidic, p*I* 4.5, 64 kDa	–	Borkow et al. (1993)
	BH2	acidic, p*I* 5.2, 26 kDa	–	Borkow et al. (1993)
	BH3	acidic, p*I* 5.0, 55 kDa	–	Borkow et al. (1993)
	BaH4	acidic, p*I* 5.3, 69 kDa	–	Franceschi et al. (2000)
	basparin A	acidic, 70 kDa	–	Loría et al. (2003)
Serine proteinase				
	asperase	acidic, 30 kDa	–	Aragón-Ortiz and Gubenšek (1978)
	ficozyme	acidic, 25 kDa	–	Fořtová et al. (1990)
	thrombin-like	acidic, 27 kDa	Q072L6	Pérez et al. (2008)
L-amino acid oxidase				
	Laao 1	acidic, 125 kDa	–	Umaña (1982a)
	Laao 2a	acidic, 125 kDa	–	Umaña (1982a)
	Laao 2b	acidic, 125 kDa	–	Umaña (1982a)
C-type lectin-like				
	aspercetin	acidic, 30 kDa	–	Rucavado et al. (2001)
Disintegrin				
	bothrasperin	acidic, 8 kDa	–	Pinto et al. (2003)

[a] Amino acid sequence entry code in UniProtKB/TrEMBL, and Protein Data Bank (PDB) entry code.