Chapter 209

Bothrops asper Metalloproteinase BaP1

Databanks

MEROPS name: BaP1 peptidase (*Bothrops asper*)
MEROPS classification: clan MA, subclan MA(M),family M12, subfamily M12B, peptidase M12.311
Tertiary structure: Available
Species distribution: known only from *Bothrops asper*
Reference sequence from: *Bothrops asper* (UniProt: P83512)

Name and History

BaP1 is a metalloproteinase from the venom of the snake *Bothrops asper* (family Viperidae, subfamily Crotalinae; common name 'terciopelo') which exerts various toxic activities, including hemorrhagic, myotoxic, dermonecrotic and pro-inflammatory effects. Its isolation was described by Gutiérrez *et al*. [1]. It is the most abundant metalloproteinase in this venom. Its name comes from '***B****othrops* ***a****sper* **P**roteinase **1**'. It has been used as a model to investigate the mechanism of local tissue damage and inflammation induced by snake venom hemorrhagic metalloproteinases.

Activity and Specificity

BaP1 hydrolyzes a variety of substrates such as hide powder azure, casein, azocasein, fibrinogen, fibronectin, nidogen, type IV collagen, laminin and various plasma and other extracellular matrix components [1–5]. Hydrolysis of hide powder azure is optimal at pH 8-0 [1], and the enzyme loses activity after incubation at 60°C [1]. When the enzyme was tested using a plasma-based, proteome-derived peptide library as substrate, along with mass spectrometry, results showed that the consensus selectivity sequence for the sites P4-P4′ corresponds to ESAE-LLLA [5]. BaP1 is inhibited by chelating agents (EDTA salts and 1,10-phenanthroline) [1], by the peptidomimetic inhibitor batimastat [6], and by α_2-macroglobulin [7].

Structural Chemistry

BaP1 is a 22.7 kDa enzyme comprised of 202 amino acids and is not glycosylated. Its sequence and 3D structure have been described [8,9]. It has six Cys residues involved in three disulfide bridges (Cys117-Cys197, Cys159-Cys181, Cys157-Cys164). It has the consensus sequence $H_{142}E_{143}XXH_{146}XXGXXH_{152}$, as well as the sequence $C_{164}I_{165}M_{166}$, characteristic of the 'metzincin' family of metalloproteinases. The mature protein is comprised of the metalloproteinase alone, thus belonging to the P-I class of snake venom metalloproteinases, according to the classification described by Fox & Serrano [10]. The active site cleft separates a major subdomain (residues 1–152), comprising four α-helices and a

five-stranded β-sheet, from the minor subdomain, which is formed by a single α-helix and several loops. The catalytic zinc ion is coordinated by the $N_{\varepsilon 2}$ atoms of His 142, His 146, and His 152, in addition to a solvent water molecule bound by Glu143 [8,9]. A loop comprising residues 153 to 176 shows flexibility [9], and this region has been proposed as playing a key role in the ability of this enzyme to induce hemorrhage [8,9,11].

Preparation

BaP1 is purified from the venom of adult specimens of *B. asper*, collected in the Pacific region of Costa Rica, by ion-exchange chromatography on CM-Sephadex, followed by gel filtration on Sephacryl S-200 and affinity chromatography on Affi-gel blue [1,12]. Owing to regional variations in the composition of *B. asper* venom between Pacific and Caribbean populations of *B. asper* in Costa Rica [13], this protocol applies only to venoms from specimens collected in the Pacific region.

Biological Aspects

BaP1 exerts several pathological and pro-inflammatory activities. Owing to its abundance in the venom, it plays a leading role in the local pathological alterations that characterize envenomations by *B. asper* [14]. It has a relatively weak hemorrhagic activity, as compared to other hemorrhagic metalloproteinases isolated from this venom [1,15]; however, due to its high concentration in the venom, it is a significant hemorrhagic component in *B. asper* venom. The mechanism of hemorrhagic activity by BaP1 has been explored and a unifying hypothesis has been presented, based on a two-step model: initially, the enzyme degrades key structural components in the basement membrane of capillary vessels and on surrounding extracellular matrix proteins which provide stability to microvessels. Then, such mechanical weakening of the capillary wall provokes hemodynamic biophysical forces, which depend on blood flow, to induce a distention and rupture of capillary wall integrity, with consequent extravasation [16,17]. The local hemorrhagic activity of BaP1 is potentiated by the drop in platelet counts induced by aspercetin, a C-type lectin-like heterodimeric protein isolated from this venom [18]. BaP1 induces myonecrosis and edema, and contributes to a deficient process of skeletal muscle regeneration [2]. It also induces dermonecrosis and blistering [12,19]. When incubated with endothelial cells in culture, in provokes detachment of cells and apoptosis [20], in addition to other mechanisms of cytotoxicity [21]. The proteomic analysis of wound exudate generated after intramuscular injection of BaP1 in mice reveals the presence of several extracellular matrix proteins, cell-membrane associated proteins and plasma proteins [4]. BaP1 elicits a prominent inflammatory activity characterized by complement activation [22] and leukocyte recruitment regulated by LECAM, CD-18 and LFA-1 adhesion molecules [23]. It promotes increments in cytokines IL-1β and IL-6, as well as matrix metalloproteinase-9 (MMP-9) in muscle [24]. In addition, it causes joint hypernociception, mediated by local release of prostaglandin E_2 and TNF-α [25], thus contributing to the pain which characterizes *B. asper* envenomations. In contrast to the prominent role played by BaP1 in the local pathological and inflammatory reaction induced by *B. asper* venom, its role in systemic pathology is limited, since intravenous injection does not induce systemic hemorrhage or lethality [1,7], probably because it is readily inhibited by the plasma proteinase inhibitor α_2-macroglobulin [7].

Distinguishing Features

Rabbit polyclonal antibodies against BaP1 have been raised and used in the detection of cross-reactive proteins in the venoms of viperid snakes [26]. Various monoclonal antibodies against BaP1 have been produced and their ability to neutralize proteolytic and hemorrhagic activity was tested [27]. These antibodies are not available on a commercial basis.

Related Peptidases

Several viperid snake venoms contain components that cross-react immunologically with BaP1 [26]. This enzyme has high sequence identity with a number of P-I metalloproteinases from viperid venoms [8]. A highly similar enzyme was characterized from the venom of *B. atrox* from Colombia [28].

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José María Gutiérrez

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica. Email: jose.gutierrez@ucr.ac.cr

Alexandra Rucavado

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica. Email: alexandra.rucavado@ucr.ac.cr

Teresa Escalante

Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica. Email: teresa.escalante@ucr.ac.cr

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