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Crystallization and preliminary diffraction data of BaP1, a haemorrhagic metalloproteinase from *Bothrops asper* snake venom

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BaP1 is a metalloproteinase isolated from the venom of the Central American snake *Bothrops asper* (terciopelo). It is a 24 kDa protein consisting of a single chain which includes the metalloproteinase domain only, therefore being classified as a class P-I snake-venom metalloproteinase. BaP1 induces prominent local tissue damage, such as haemorrhage, myonecrosis, blistering, dermonecrosis and oedema. In order to elucidate its structure, BaP1 was crystallized by the hanging-drop vapour-diffusion technique in 0.1 *M* bicine pH 9.0, 10% PEG 20 000 and 2%(*v*/*v*) dioxane. Diffraction data were observed to a resolution of 2.7 Å. Crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 38.22$, $b = 60.17$, $c = 86.09$ Å.

1. Introduction

Snake venoms are extremely rich sources of zinc-dependent metalloproteinases (Bjarnason & Fox, 1994). Snake-venom metalloproteinases (SVMPs) and the ADAMs (metalloproteinases comprising additional disintegrin-like, high-cysteine and transmembrane domains) constitute the 'reprolysin' subfamily of metalloproteinases which, together with matrix metalloproteinases (MMPs), astacins and serralysins, form the 'metzincin' family, which have a characteristic consensus sequence in the zinc-binding environment and the presence of a methionine turn (Bode *et al.*, 1993). SVMPs play a relevant role in the pathogenesis of viperine and crotaline snake-bite envenomations, inducing haemorrhage (Bjarnason & Fox, 1994; Gutiérrez & Rucavado, 2000). Haemorrhagic activity is one of the most relevant pathophysiological effects in envenomations by snakes of the family Viperidae, contributing to local tissue damage, cardiovascular shock and other haemodynamic alterations (Ohsaka, 1979; Bjarnason & Fox, 1994). Extravasation is a consequence of the proteolytic digestion exerted by SVMPs on components of the basal lamina, an extracellular matrix structure that supports endothelial cells in microvessels. As a result, the structural integrity of capillaries is impaired and haemorrhage ensues.

Comparative amino-acid and nucleotide-sequence studies have revealed a variable domain constitution in SVMPs (Hite *et al.*, 1994). Some of them comprise a metalloproteinase domain only (class P-I), whereas others additionally possess a disintegrin-like domain (class P-II) or disintegrin-like and high-cysteine domains (class P-III). In addition to these three domains, another group of SVMPs also presents a lectin-like subunit linked by a disulfide bridge to the main chain (class P-IV). P-III SVMPs usually display higher haemorrhagic activity than P-I SVMPs (Bjarnason & Fox, 1994). It has been proposed that the disintegrin-like and high-cysteine domains contribute to this enhanced activity either by directing the enzyme to relevant cellular targets or by inhibiting platelet aggregation, thereby precluding a normal haemostatic process (Kamiguti *et al.*, 1996).

Class P-I SVMPs, possessing only the metalloproteinase domain, present conspicuous differences in their pharmacological profiles: some induce prominent haemorrhage, whereas others are devoid of this activity, albeit being highly active as proteolytic enzymes. Thus, despite the observation that proteolysis is required to exert haemorrhage (Bjarnason & Fox, 1994), structural features in addition to the catalytic site are evidently required to induce extravasation. Structural studies have been performed on various P-I SVMPs (Gomis-Ruth *et al.*, 1993; Zhang *et al.*, 1994; Kumasawa *et al.*, 1996; Gong *et al.*, 1998). However, the structural basis of the highly variable haemorrhagic activity of class P-I SVMPs is not clear at present. In order to further characterize this group of metalloproteinases, in this communication we report the crystallization and preliminary X-ray analysis of BaP1, a class P-I SVMP with weak haemorrhagic activity isolated from the venom of the Central American crotaline snake *Bothrops asper*. This enzyme plays a relevant role in the local tissue damage associated with *B. asper* envenomation, since it induces local haemorrhage, myonecrosis, oedema, blistering, dermonecrosis and complement activation (Gutiérrez *et al.*, 1995; Rucavado *et al.*, 1995,

1998; Farsky *et al.*, 2000). The haemorrhagic and proteolytic activities of BaP1 are inhibited by chelating agents such as EDTA (Gutiérrez *et al.*, 1995).

2. Methods

2.1. Purification

BaP1 was purified from a venom pool obtained from more than 40 adult specimens of *B. asper* collected in the Pacific slopes of Costa Rica. Venom was lyophilized and stored at 253 K. Isolation was carried out by ion-exchange chromatography on CM-Sephadex, gel filtration on Sephacryl S-200 and affinity chromatography on Affi-Gel Blue, as previously described (Gutiérrez *et al.*, 1995; Rucavado *et al.*, 1998). Homogeneity was demonstrated by SDS–PAGE run under reducing and non-reducing conditions using 12% acrylamide gels (Laemmli, 1970). A single band of 24 kDa was observed in the gels.

2.2. Crystallization

A lyophilized sample of BaP1 was dissolved in doubly distilled water at a concentration of 10 mg ml^{-1}. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture plates. Initial trials were carried out with a screen similar to that described by Jancarik & Kim (1991). Typically, 1 µl drops of protein solution were mixed with an equal volume of the screening solution and equilibrated over 1 ml of the latter as reservoir solution. The conditions were refined by trial and error and single crystals ($\sim$0.1 $\times$ 0.06 $\times$ 0.04 mm) were obtained when a 2 µl protein droplet was mixed with an equal volume of reservoir solution consisting of 0.1 *M* bicine pH 9.0, 10% PEG 20 000 and 2%(*v*/*v*) dioxane.

2.3. X-ray diffraction data collection

Crystals of BaP1 were mounted in thin-walled glass capillaries and were examined with Cu $K\alpha$ radiation generated by a Rigaku RU-200 rotating-anode generator operating at 50 kV and 90 mA equipped with Osmic mirrors. Diffraction intensities were measured at room temperature using an R-AXIS IV^{+} imaging-plate detector at a distance of 150 mm from the crystal with an oscillation range of 2° per image. The crystals diffracted X-rays to a maximum resolution of 2.7 Å. A complete data set was obtained from 64 frames using the program *DENZO* and the data were scaled and reduced using *SCALEPACK* (Otwinowski & Minor, 1997). Data-processing statistics are presented in Table 1.

3. Results

The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 38.22, b = 60.17, c = 86.09 Å, as judged from autoindexing and consideration of systematically absent reflections. Assuming there to be one molecule of BaP1 in the asymmetric unit, a Matthews parameter value (Matthews, 1968) of 2.06 Å^3 Da^{-1} was obtained with an approximate solvent content of 46%, values that are within the expected range for typical protein crystals. The plate-like crystals diffracted to 2.7 Å resolution in two dimensions but only to 3.2 Å in the third dimension. Processing of the 38 443 measured reflections led to 5511 unique reflections, with an $R_{\rm merge}$ of 16.2% for the data to 2.7 Å resolution. Processing the data to a maximum resolution of 3.2 Å led to an $R_{\rm merge}$ of 8.7% for 3384 unique reflections (Table 1).

Molecular-replacement studies were carried out using the program *AMoRe* (Navaza, 1994) using a homology-built model for metalloproteinase BaP1 which was based on the crystal structure of adamalysin II (PDB code 1iag; Gomis-Ruth *et al.*, 1993), a class P-I SVMP from *Crotalus adamanteus*. Data in the resolution range 20–3.5 Å were used. A solution was obtained for the rotation and translation functions assuming one molecule in the asymmetric unit, leading to a final correlation coefficient of 55.3% and an R factor of 43% after rigid-body refinement for data in the resolution range 20–3.5 Å. Using the atomic coordinates of atrolysin C (PDB code 1atl; Zhang *et al.*, 1994), the correlation coefficient and R factor were 56.4 and 43.8%, respectively, after rigid-body refinement using data in the same resolution range. The structure refinement will be completed when the amino-acid sequence, which is currently being determined, becomes available.

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Table 1
Data-collection and processing statistics.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 38.22, b = 60.17, c = 86.09
Maximum resolution (Å)	2.7
Resolution of data set (Å)	28.4–3.2
No. of unique reflections	3384
$R_{\rm merge}$† 3.2 Å (2.7 Å) (%)	8.7 (16.1)
Completeness (%)	97.0
$V_{\rm M}$ (Å^3 Da^{-1})	2.06
No. of molecules per asymmetric unit	1
$I/\sigma(I)$ in outermost shell, 3.4–3.2 Å (2.8–2.7 Å)	3.6 (1.5)

† $R_{\rm merge} = \sum\sum|I(h)_i - \langle I(h)\rangle|/\sum\langle I(h)\rangle$, where $I(h)$ is the observed intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean intensity of reflection h calculated after scaling.

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