Acta Crystallographica Section F
Biology
ISSN 1744-3091

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Received 10 December 2005
Accepted 20 February 2006

Blotched snakehead virus (BSNV) is a member of the *Birnaviridae* family that requires a virally encoded protease known as VP4 in order to process its polyprotein into viral capsid protein precursors (pVP2 and VP3). VP4 belongs to a family of serine proteases that utilize a serine/lysine catalytic dyad mechanism. A mutant construct of VP4 with a short C-terminal truncation was overexpressed in *Escherichia coli* and purified to homogeneity for crystallization. Using the sitting-drop vapour-diffusion method at room temperature, protein crystals with two distinct morphologies were observed. Cubic crystals grown in PEG 2000 MME and magnesium acetate at pH 8.5 belong to space group *I*23, with unit-cell parameters $a = b = c = 143.8$ Å. Trigonal crystals grown in ammonium sulfate and glycerol at pH 8.5 belong to space group *P*321/*P*312, with unit-cell parameters $a = b = 158.2$, $c = 126.4$ Å.

1. Introduction

Blotched snakehead virus (BSNV) was first identified in cell lines derived from the blotched snakehead (*Channa punctata*), a warm-water fish of south-east Asia (John & Richards, 1999). It is a member of the family *Birnaviridae*, which includes three genera, *Aquabirnavirus*, *Avibirnavirus* and *Entomobirnavirus*, that infect fish, birds and insects, respectively (Delmas *et al.*, 2005; http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm). The prototype members of this virus family, infectious pancreatic necrosis virus (IPNV) for *Aquabirnavirus* and infectious bursal disease virus (IBDV) for *Avibirnavirus*, are well known causative agents of economically important diseases affecting

proteases whose crystal structures have been solved include signal peptidase (Paetzel *et al.*, 1998, 2002, 2004), UmuD′ (Peat *et al.*, 1996), LexA (Luo *et al.*, 2001), λ repressor (Bell *et al.*, 2000), D-Ala-D-Ala carboxypeptidase (Thunnissen *et al.*, 1995), Lon protease (Botos *et al.*, 2004, 2005; Im *et al.*, 2004) and the C-terminal processing peptidase (Liao *et al.*, 2000).

In this report, we describe the DNA manipulation, overexpression and crystallization of a C-terminal truncated version of BSNV VP4 (amino acids 558–773). The VP4 construct did not encode an affinity tag for purification and therefore a series of purification steps were necessary to obtain a homogeneous protein sample for crystallization. Upon screening for initial crystallization conditions, two different crystal forms were identified and optimized for X-ray diffraction. The structure of the *Birnaviridae* viral proteases has not been studied previously by crystallographic means and to our knowledge this is the first crystallization report of a Ser-Lys protease of viral origin. The BSNV VP4 structure is expected to reveal the configuration of a Ser-Lys catalytic dyad that may be structurally conserved in other genera of the *Birnaviridae*.

2.

2.1.

An expression construct encoding the entire open reading frame of BSNV VP4 (amino acids 558–791) in pET-28b+ (Novagen) using *Nde* I and *Bam*HI sites was created as previously described (Da Costa *et al.*, 2003). A deletion mutant construct that removed 18 amino acids at the C-terminus was created by converting the codon for Pro774 of the original plasmid template to a termination codon. The required site-directed mutagenesis was carried out using the Quick Change kit (Stratagene) with forward primer 5′-GGAGTCAACAGTCACCCG-CTGAGAGAGCGTCGGACACCAG-3′ and reverse primer 5′-CT-GGTGTCCGACGCTCTCTCAGCGGGTGACTGTTGACTCC-3′. This new construct was confirmed by DNA sequencing. For over-expression, the new plasmid was transformed into *Escherichia coli* strain Tuner (DE3) and the transformants were selected on LB agar plates containing 0.05 mg ml^{-1} kanamycin. From a single colony, an overnight culture (100 ml) was grown at 310 K in LB media supplemented with 0.05 mg ml^{-1} kanamycin. The overnight culture was used to inoculate 1 l of the same media in a 1/100 dilution and the culture was induced with 0.5 m*M* IPTG at mid-log growth phase and grown for an additional 3 h at 310 K. The cells were harvested by centrifugation at 5000 for 15 min n and

molecular weights were calculated using the Debye fit method (Andersson *et al.*, 2003).

2.4. [illegible]

Crystallization was carried out by the sitting-drop vapour-diffusion method at 294 K. 1 µl protein sample and 1 µl reservoir were mixed thoroughly and equilibrated against 1 ml of reservoir solution. The initial crystallization conditions were screened using commercial sparse-matrix (Jancarik & Kim, 1991) and PEG/Ion screen kits (Hampton Research). The protein was found to crystallize under several different conditions within 4–7 d. Two distinctly different conditions that generated crystals with different morphology were optimized for size and uniformity. Crystals with a polyhedral appearance (Fig. 2*a*) were obtained in 0.1 *M* Tris–HCl pH 8.5, 25% PEG 2000 MME (polyethylene glycol monomethyl ether) and 40 m*M* magnesium acetate. Hexagonal shaped crystals (Fig. 2) were obtained in 0.1 *M* Tris–HCl pH 8.5, 1.65 *M* ammonium sulfate and 20% glycerol.

2.5. [illegible]