Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction studies of *Murraya koenigii* trypsin inhibitor

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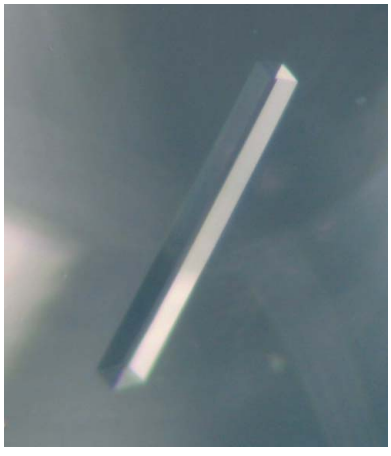
Received 3 January 2007
Accepted 12 March 2007



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A Kunitz-type trypsin inhibitor purified from the seeds of *Murraya koenigii* has been crystallized by the sitting-drop vapour-diffusion method using PEG 8000 as the precipitating agent. The crystals belong to the tetragonal space group $P4_32_12$, with unit-cell parameters $a = b = 75.8$, $c = 150.9$ Å. The crystals contain two molecules in the asymmetric unit with a V_M value of 2.5 Å^3 Da^{-1}. Diffraction was observed to 2.65 Å resolution and a complete data set was collected to 2.9 Å resolution.

1. Introduction

Trypsin inhibitors are widely distributed in nature and have been isolated from many sources, including plants (Laskowski & Kato, 1980; Mosolov & Valueva, 1993). Plant seeds are a rich source of inhibitors, which have been described as endogenous regulators of proteolytic activity (Ryan, 1991) and as storage proteins (Xavier-Filho, 1992). Plant proteinase inhibitors have also been described as natural plant defence agents (Shewry & Lucas, 1997; Shukle & Wu, 2003). The proteinase inhibitors play an important role in regulating many physiological processes such as inflammation, coagulation, fibrinolysis, complement-activation intracellular protein breakdown, the cell cycle, transcription and apoptosis (Silverman *et al.*, 2001). Most plant serine proteinase inhibitors have been characterized from seeds of the Leguminosae, Cucurbitaceae, Solanaceae and Graminae families (Garcia-Olmeda *et al.*, 1987). They have been classified according to their sequence homology and the location of disulfide bonds. These include Kunitz-type, Bowman–Birk type, potato I, potato II, squash, cereal superfamily and thaumatin-like inhibitors. The Kunitz family of trypsin inhibitors are proteins of MW $\simeq$ 20 kDa with four cysteine residues that form two disulfide bonds (Richardson, 1991).

A Kunitz-type trypsin inhibitor was purified from the seeds of *Murraya koenigii* (curry-leaf tree), which belongs to the Rutaceae family. *M. koenigii* trypsin inhibitor (MKTI) is a single-chain protein. The approximate molecular weight of the protein was determined to be 27 kDa from SDS–PAGE analysis. However, MALDI–TOF analysis revealed a molecular weight of 21.4 kDa (unpublished results). The purified protein inhibited bovine pancreatic trypsin at a molar ratio of 1:1.1 with a dissociation constant of 7×10^{-9} *M*. The maximum inhibition was observed at pH 8.0. Solubility studies under various pH conditions showed that the inhibitor is soluble at and above pH 7.5 and slowly precipitates below this pH. The N-terminal sequence of the first 15 amino acids showed a significant resemblance, with 72% identity, to a Kunitz-type *Erythrina variegata* chymotrypsin inhibitor (ECI) precursor (Shee & Sharma, 2007). We report here the crystallization and preliminary X-ray diffraction analysis of the MKTI purified from *M. koenigii* seeds.

2. Purification

Purification of the protein was performed as described previously (Shee & Sharma, 2007). Briefly, *M. koenigii* seeds were crushed and soaked overnight at 277 K in 30 ml 50 m*M* Tris–HCl buffer pH 7.5. The homogenate was cleared by centrifugation at 12 000*g* for 1 h and the supernatant was loaded onto a DEAE Sepharose column

equilibrated with the same buffer as used for sample extraction. The unbound molecules were washed extensively and bound molecules were eluted with NaCl in a step gradient from 0 to 500 m*M* in the same buffer. Fractions with trypsin inhibitory activity were pooled and concentrated. The protein was further purified by HPLC using a size-exclusion chromatography column. The purity of the protein was determined by 15% SDS–PAGE analysis (Laemmli, 1970). The protein concentration was estimated by a standard dye-binding method using bovine serum albumin (Sigma) as the standard (Marion, 1976) and by measuring the absorbance at 280 nm.

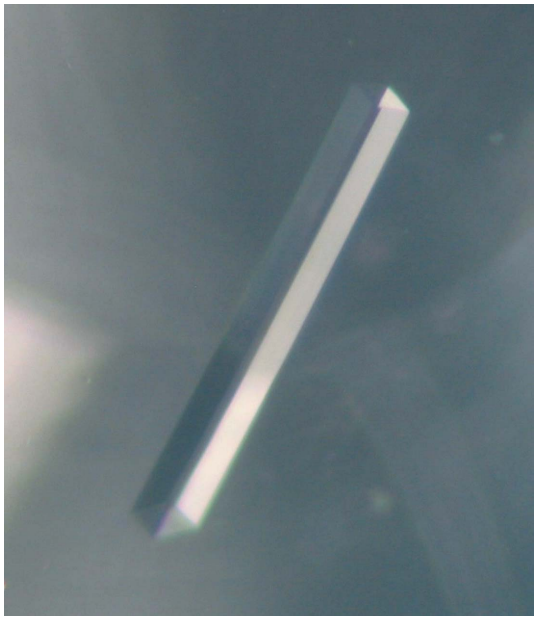

Figure 1
Crystal of *M. koenigii* trypsin inhibitor as grown by the sitting-drop method. The dimensions of the crystal were 0.10 × 0.10 × 0.75 mm.

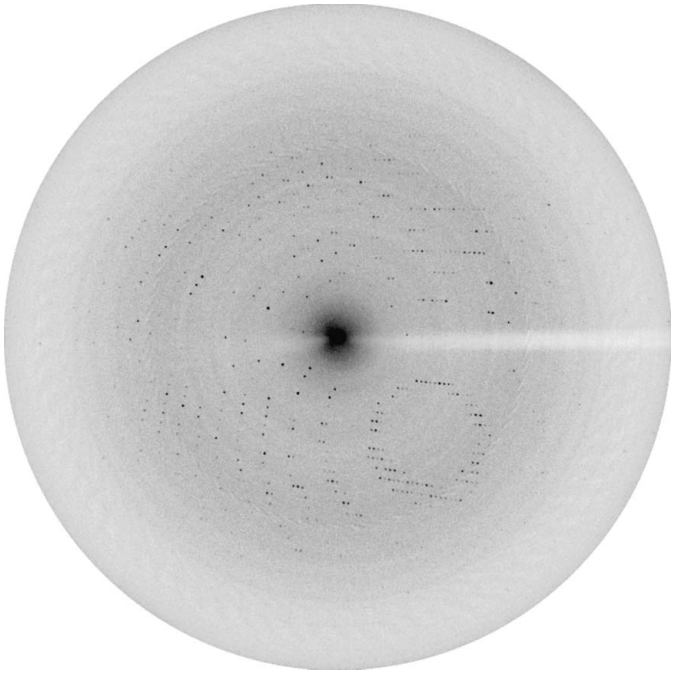

Figure 2
X-ray diffraction image from a *M. koenigii* trypsin inhibitor crystal. The crystal diffracted to 2.65 Å resolution.

Table 1
Crystal data and intensity statistics.

Values in parentheses are for the last resolution shell.

Space group	$P4_32_12$
Unit-cell parameters (Å)	$a = b = 75.8$, $c = 150.9$
Temperature (K)	293
Resolution range (Å)	25–2.9 (2.95–2.9)
No. of observed reflections	58581
No. of unique reflections	10300 (540)
Completeness	96.1 (99.2)
R_{sym}† (%)	14.6 (42.2)
Mean $I/\sigma(I)$	4.7 (1.6)

† $R_{sym} = \sum_{hkl,i} |I_i - \langle I \rangle| / \sum_{hkl,i} I_i$.

3. Crystallization and data collection

Crystallization experiments were performed using the sitting-drop vapour-diffusion method at 293 K. Crystals of MKTI were grown in 4 µl drops containing equal volumes of protein (25 mg ml^{-1} in 100 m*M* Tris–HCl pH 8.0) and reservoir solution. The reservoir solution consists of 6%(*w*/*v*) PEG 8000, 100 m*M* Tris–HCl buffer pH 8.0, 80 m*M* NaCl, 80 m*M* $CaCl_2$ and 8%(*v*/*v*) glycerol. Crystals of MKTI appeared in 4 d and grew to maximum dimensions of 0.10 × 0.10 × 0.75 mm in 10 d (Fig. 1). A single crystal was mounted in a 0.5 mm capillary using reservoir buffer. Diffraction data were collected on a MAR345 imaging-plate system using Cu *Kα* radiation generated by a Rigaku rotating-anode generator at the Biophysics Department, All India Institute of Medical Sciences, New Delhi, India. Data collection was performed at 293 K with a crystal-to-detector distance of 220 mm and 1° oscillation per image (Fig. 2). The data were processed with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Based on the molecular weight of MKTI (21.4 kDa) and space group $P4_32_12$, it is assumed that the crystal contains two molecules per asymmetric unit. This assumption gives a V_M value (Matthews, 1968) of 2.5 Å^3 Da^{-1} and a solvent content of 51%. The data-collection statistics are summarized in Table 1.

We thank Dr Punit Kaur, Mrs Asha Bhushan and Mr Hidayat for help in data collection and processing. CS thanks the Ministry of Human Resource Development, Government of India for financial support.

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