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Crystallization and preliminary crystallographic study of cucurmosin, a ribosome-inactivating protein from the sarcocarp of *Cucurbita moschata*

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Cucurmosin, a ribosome-inactivating protein purified from pumpkin, the sarcocarp of *Cucurbita moschata*, has been crystallized using polyethylene glycol as a precipitant. The crystals belong to space group $P2_12_12_1$ and have unit-cell parameters a = 41.91, b = 59.48, c = 98.78 Å. There is one molecule in the asymmetric unit. The diffraction data to 3.0 Å resolution were collected on a MAR Research image-plate detector.

1. Introduction

Many plants contain ribosome-inactivating proteins (RIPs) which are RNA N-glycosidases (Barbieri *et al.*, 1993; Endo *et al.*, 1988). RIPs cleave the N-glycosidic bond of a single adenine at position A4324 in the 28S rRNA of rat. Because of the removal of one adenine base from rRNA, elongation factor II (EF-2) cannot bind to the 60S subunit of eukaryotic ribosomes, with a consequent inhibition of protein synthesis. RIPs are conventionally classified into two types: type I and type II RIPs. Type I RIPs are monomeric proteins with molecular weights of about 30 kDa. Most RIPs isolated from cucurbiteceae plants belong to type I, such as trichosanthin, momorcharin, luffin and bryodin. Type II RIPs are heterodimeric proteins consisting of an enzymatically active *A* chain and a lectin-like *B* chain; ricin and abrin belong to type II. By comparing the amino-acid sequences of ten kinds of RIP and the crystal structures of trichosanthin (Pan *et al.*, 1993, 1994) and ricin (Katzin *et al.*, 1991), we noticed that the *A* chain of type II RIPs is similar to type I RIPs.

RIPs have been of considerable interest in recent years owing to their antitumour and antivirus activities. Moreover, some type I RIPs from cucurbitaceae, trichosanthin and TAP29 (McGrath *et al.*, 1989; Lee-Huang *et al.*, 1991), momorcharin and MAP30 (Yeung *et al.*, 1986; Lee-Huang *et al.*, 1990) and bryodin (Michael *et al.*, 1993) have also been shown to have potent activity against HIV-1 infected T-cells and macrophages. All these have stimulated further interest in determining the structure–function relationship in RIPs.

A new RIP, cucurmosin, isolated from pumpkin, the sarcocarp of *C. moschata*, a member of the cucurbiteceae family, was recently purified by an improved procedure and its molecular mass was determined to be about 28 kDa (Chen & Ye, unpublished results). To date, more than 60 type I RIPs have been purified from a variety of plants and their different organs, such as seeds, leaves, roots and latex, but only two RIPs have been found in the sarcocarp of any fruit or edible gourd in China and worldwide (Yoshinari *et al.*, 1996). We are studying the structure and function of cucurmosin, as it is important in nutrition, physiology and pharmacology.

Two crystal structures of type I RIPs from the cucurbiteceae family have been reported: trichosanthin (Pan *et al.*, 1993; Zhou *et al.*, 1994; Lin *et al.*, 1997) and momorcharin (Husain *et al.*, 1994). The three-dimensional structure of type I RIPs is similar to that of the *A* chain of type II RIPs, according to the least-squares superposition of their C^{α}-atom traces (Monzingo *et al.*, 1993; Zhou *et al.*, 1994). In our research work on the structure of type I RIPs, we have recently obtained crystals of cucurmosin.

2. Experiment

The purified protein solution showed only one band on SDS–PAGE and was concentrated using an Ultrafree-CL filter (Millipore). Crystallization was performed by the hanging-drop vapour-diffusion method at room temperature (McPherson, 1982). A number of different conditions, involving different buffer-solution systems, pH values, precipitants and protein concentrations, were systematically examined for their effects on crystallization. The crystals of cucurmosin were obtained in citrate buffer or phosphate buffer with polyethylene glycol as precipitant.

The optimized crystallization conditions are as follows. The reservoir solution contained 100 mmol l^{-1} phosphate buffer pH 7.5, 30%(*v*/*v*) polyethylene glycol (PEG 6000). The droplet consisted of equal volumes of protein solution (20 mg ml^{-1}) and reservoir solution. Crystals of dimensions 0.4 × 0.2 × 0.1 mm appeared after six months.

Table 1
Data-collection statistics.

Resolution shell (Å)	Number of unique reflections	Completeness (%)	R_{merge}† (%)
100.00–7.40	387	93.0	0.080
7.40–5.87	365	98.4	0.103
5.87–5.13	357	98.6	0.114
5.13–4.66	349	98.3	0.110
4.66–4.33	349	97.8	0.113
4.33–4.07	345	96.6	0.122
4.07–3.87	341	96.3	0.133
3.87–3.70	340	98.3	0.144
3.70–3.56	331	95.9	0.156
3.56–3.43	343	96.1	0.173
3.43–3.33	328	98.2	0.170
3.33–3.23	328	94.5	0.213
3.23–3.15	337	98.5	0.221
3.15–3.07	336	94.6	0.228
3.07–3.00	319	96.7	0.246
All *hkl*	5155	96.8	0.139

A crystal was mounted in a thin-walled glass capillary and the capillary was sealed with wax after filling both ends with mother liquor. X-ray data were collected on a MAR Research IP300 detector. The crystals belong to the orthorhombic system, with unit-cell parameters a = 41.91, b = 59.48, c = 98.78 Å. The systematic absences revealed the space group to be $P2_12_12_1$.

Assuming one molecule in the asymmetric unit, the V_m (Matthews, 1968) value is calculated to be 2.20, which indicates that the crystal contains 44% solvent. A data set at 3.0 Å resolution has been collected; data-collection statistics are listed in Table 1. The high R_{merge} values show that the crystal is slightly twinned.

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