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Crystallization and preliminary X-ray analysis of luffaculin, a ribosome-inactivating protein from sponge-gourd seeds

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Luffaculin is a ribosome-inactivating protein. Crystals suitable for X-ray diffraction were first obtained using the hanging-drop vapour-diffusion method. X-ray studies show that the crystals belong to space group $C2$, with unit-cell parameters $a = 89.90$, $b = 59.82$, $c = 55.18$ Å, $\beta = 120.81^\circ$, and have one molecule in the crystallographic asymmetric unit. The crystals diffract X-rays to at least 2.0 Å resolution.

1. Introduction

Many plants contain at least one type of ribosome-inactivating protein (RIP; Barbieri & Stirpe, 1982; Stirpe & Barbieri, 1986; Roberts & Claude, 1986). RIPs inhibit the protein synthesis of eukaryotic cells by cleaving a single adenine base from a highly specific site on the 28S RNA of the 60S ribosomal subunit (Endo & Tsurugi, 1987). RIPS have attracted attention as having potential application in the treatment of diseases such as cancer and AIDS owing to their cellular toxicity. There are two types of RIPs (Stirpe & Barbieri, 1986). Type I proteins are single chained, whereas type II proteins are double chained. The A chain of the type II proteins possesses the ribosome-inactivating property; the B chain is responsible for attaching the protein molecule to the target-cell surface in order to assist the A chain in crossing the cell membrane. Type II RIPs are, therefore, among the most toxic cytotoxins. Trichosanthin and momorcharin belong to the type I RIPs, while ricin and abrin belong to type II. The crystal structures of both trichosanthin (Gao *et al.*, 1993) and ricin (Montfort *et al.*, 1987) have been elucidated. Trichosanthin and ricin A are not only homologous in amino-acid sequence (Zhang & Wang, 1986), but are also similar in three-dimensional structure. Since trichosanthin and ricin are from taxonomically distant species, *Trichosanthes kirilowii* of the Cucurbitaceae family and *Ricinus communies* of the Euphorbiaceae family, respectively, it appears that the widely distributed RIPs of both types must originate from the same ancestor and assume the same 'RIP fold'.

Luffaculin is classified as a type II RIP. Despite the fact that luffaculin is a glycoprotein, whereas trichosanthin contains no carbohydrates, they share many common features. Like almost all type II RIPs (Stirpe & Barbieri, 1986), they have a comparable molecular mass (26–31 kDa) and a strongly basic pI (∼pH 9). They induce mid-term abortion in pregnant mice and inhibit cell-free protein synthesis with similar potency (Yeung *et al.*, 1991). Although the primary structure of luffaculin is as yet unknown, we assumed luffaculin to have a similar spatial structure to trichosanthin. We used molecular-replacement methods to determine the structure of luffaculin, using trichosanthin as a model, and obtained a preliminary solution.

2. Crystallization

The proteins used for crystallization were extracted from sponge-gourd seeds (*Luffa acutangla* from Guangxi province, China). The purification of the proteins referred to that of luffin A (Kamenosono *et al.*, 1988; Wu *et al.*, 1995). The counterpart of luffin A was collected, which inhibits protein synthesis in a cell-free system (rabbit reticulocyte lysate). Crystals were obtained by the hanging-drop vapour-diffusion method. The crystallization protocol involved mixing 5 µl reservoir solution [0.05 *M* Tris–HCl pH 7.5, 40%(*w*/*v*) $(NH_4)_2SO_4$] with 5 µl 40 mg ml^{-1} protein solution [0.15 *M* NaCl, 0.1%(*w*/*v*) NaN_3] to form a hanging drop that was allowed to equilibrate with the reservoir solution at room

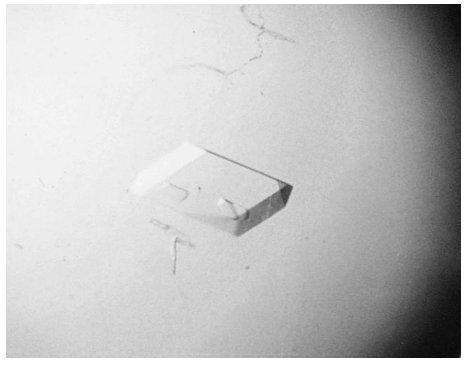

Figure 1
Crystals of luffaculin.

Table 1
X-ray diffraction data.

Number of observed reflections	50139
Number of unique reflections	16809
Resolution range (Å)	
Overall	20.00–2.0
Outermost shell	2.07–2.0
$I > 2\sigma(I)$ (%)	
Overall	81.3
Outermost shell	52.8
Completeness (%)	
Overall	98.8
Outermost shell	97.4
$R_{\rm merge}$ (%)	
Overall	9.7
Outermost shell	43.9
Multiplicity	3.0

temperature. Crystals grew to a final size of about 0.8 × 0.4 × 0.1 mm (Fig. 1).

3. Data collection

Three-dimensional intensity data were collected to 2.0 Å resolution at room temperature on a MAR Research image plate (300 mm) with a Rugaku RU-200 rotating copper anode generator operating at 40 kV and 100 mA. The crystal-to-detector distance was 135 mm. The data were collected in 1.5° oscillation frames over a 180° oscillation range. The data were processed using the *DENZO* program (Table 1). The crystals belong to space group *C*2, with unit-cell parameters a = 89.90, b = 59.82, c = 55.18 Å, β = 120.81°, and have one molecule in the asymmetric unit. The value of V_m is 2.75 Å^3 Da^{-1}, corresponding to a solvent content of 55% (Matthews, 1968).

Preliminary molecular-replacement calculations were carried out using the program *AMoRe* (Navaza, 1994), using diffraction data between 8 and 3.5 Å resolution and the atomic coordinates of trichosanthin as a search model. After rotation and translation calculations, one clear solution was obtained with a correlation coefficient of 38.4% (next highest value 12.5%) and a crystallographic R factor of 46.2%. After rigid-body refinement, the correlation coefficient was 45.4% and the R factor was 44.1%. Final structure determination is in progress.

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