Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

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

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Received 15 April 1999
Accepted 1 November 1999

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.

This work is supported by the National Natural Science Foundation of China (No. 39900025). We give our heartfelt thanks to Mr Hu Yugang, Dr Chai Jijie, Dr Zhang Hailong and Dr Guan Rongjin for their help.

References

Barbieri, L. & Stirpe, F. (1982). *Cancer Surv.* **10**, 489–520.
Endo, Y. & Tsurugi, K. (1987). *J. Biol. Chem.* **262**, 8128–8130.
Gao, B., Ma, X.-Q., Wang, Y.-P., Wu, S., Chen, S.-Z. & Dong, Y. C. (1993). *Sci. China (Ser. B)*, **23**, 272–282.
Kamenosono, M., Nishida, H. & Funatsu, G. (1988). *Agric. Biol. Chem.* **52**(5), 1223–1227.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Montfort, W., Villafranca, J. E., Monzingo, A. F., Ernst, S. R., Katzin, B., Rutenber, E., Xuong, N. H., Hamlin, R. & Robertus, J. D. (1987). *J. Biol. Chem.* **262**, 5398–5403.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Roberts, W. K. & Claude, P. S. E. (1986). *Biosci. Rep.* **6**, 19–29.
Stirpe, F. & Barbieri, L. (1986). *FEBS Lett.* **195**, 1–8.
Wu, S., Zhu, Y. R., Guo, F. & Liu, D. H. (1995). *Prog. Biochem. Biophys.* **22**(5), 464–468.
Yueng, H. W., Li, W. W. & Ng, T. B (1991). *Int. J. Pept. Protein Res.* **38**, 15–19.
Zhang, X.-J. & Wang, J.-H. (1986). *Nature (London)*, **321**, 477–478.