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Crystallization of agglutinin from the seeds of *Abrus precatorius*

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Agglutinin protein purified from the seeds of *Abrus precatorius* has a high antitumour activity and was crystallized at room temperature with polyethylene glycol 8000 as the precipitant. The agglutinin crystal diffracted to 3.45 Å and belongs to one of two possible tetragonal space groups, $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 141.91$, $c = 105.63$ Å. The asymmetric unit contains a heterotetrameric protein molecule of molecular weight 134 kDa and has a solvent content of approximately 38%.

1. Introduction

The plant *A. precatorius*, which has decorative seeds, is widely distributed in almost all tropical and subtropical regions. It contains at least two closely related lectins, the cytotoxic abrin and the related non-toxic *A. precatorius* agglutinin (AAG), which were found to exhibit antitumour activity in animal tests (Lin *et al.*, 1970) and were isolated from the seeds in our laboratory (Lin *et al.*, 1980). Both abrin and AAG have very pronounced antitumour activity.

Abrin is a heterodimer consisting of a toxophore *A* chain linked to a cell-binding *B* chain by a disulfide bond (Olsnes & Phil, 1973). Four isoabrins, abrin-a, abrin-b, abrin-c and abrin-d, were purified by us and shown to be highly toxic (Lin *et al.*, 1980). The very high toxicity of abrin was considered to be the result of a strong inhibitory effect on protein biosynthesis. Its median lethal dose (LD_{50}) is 12 µg per kilogram body weight (Lin *et al.*, 1970), in contrast to the greater than 5 mg per kilogram body weight for AAG (Lin *et al.*, 1980).

AAG, a glycoprotein, is a tetramer consisting of two *A* (AAG *A*) and two *B* (AAG *B)* peptide chains linked by disulfide bonds (Olsnes & Phil, 1973). The molecular weights of AAG, AAG *A* and AAG *B* are about 134, 32 and 35 kDa, respectively (Lin *et al.*, 1980). The minimum concentration of AAG leading to erythrocyte haemagglutination is 30 µg per kilogram body weight (Lin *et al.*, 1980). AAG is also a protein-synthesis and DNA-synthesis inhibitor *in vitro*. *In vivo*, AAG can also inhibit the growth of Ehrlich ascites tumour cells, meth-A cancer cells and sarcoma 180 cells (Lin *et al.*, 1969; Tung *et al.*, 1976). The life span of mice inoculated intraperitoneally with tumour cells increased significantly on treatment with 2.0 µg per kilogram body weight of AAG compared with control groups (Lin *et al.*, 1981). In addition, AAG was shown to possess mitogentic activity when tested on human lymphocytes (Tung *et al.*, 1976). These biochemical functions of agglutinin attracted our interest in further investigations. X-ray structure determination was considered an effective way to proceed.

2. Materials and methods

Sepharose 6B and Sephadex G-100 were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden).

AAG was extracted by homogenizing 200 g of *A. precatorius* kernels in 1 l cold 5% acetic acid and the homogenate was centrifuged at 10 000*g* for 20 min. The supernatant was fractionated with ammonium sulfate and the proteins which precipitated between 35 and 95% ammonium sulfate were collected and dialyzed for 48 h against 0.005 *M* phosphate buffer pH 8.0. After dialysis, the extracts were centrifuged at 10 000*g* for 20 min; the supernatants are referred in the following as 'the crude extracts'.

The crude extracts obtained were purified with a Sepharose 6B affinity column (3.0 × 50 cm), which was pre-equilibrated and washed with 0.1 *M* phosphate buffer pH 8.0. The column was eluted with 0.1 *M* buffer containing 0.1 *M* D-galactose to obtain isoabrins and AAG (Lin *et al.*, 1980). Separation of isoabrins and AAG was carried out by gel filtration with a Sephadex G-100 column (2.2 × 100 cm). Two peaks were obtained: AAG was found in the first peak, while isoabrins were found in the second peak.

The method used to screen conditions for protein crystallization was that suggested by Jancarik & Kim (1991). Crystals suitable for X-ray analysis were obtained by the sitting-drop vapour-diffusion method at room temperature [296 (2) K; McPherson, 1982].

8 µl of protein solution at a concentration of 10 mg ml^{-1} prepared from lyophilized protein was mixed with 8 µl of reservoir solution containing PEG 8000; the precipitant condition was 0.1 *M* Tris pH 7.5 with 6.5% PEG 8000 and crystals appeared after nearly four months.

Fig. 1 shows a polyhedral crystal of agglutinin obtained by the present method. A crystal of dimensions 0.35 × 0.30 × 0.27 mm was mounted in a thin capillary 0.7 mm in diameter containing a small amount of mother liquid and sealed with epoxy resin. X-ray diffraction was measured with an image-plate detector (Rigaku R-AXIS IV) on a rotating-anode X-ray generator (Rigaku RU-300) at 295 (2) K using Cu $K\alpha$ radiation (50 kV and 100 mA). The crystal-to-detector distance was 150 mm. The diffraction power of the crystal was not weakened by X-ray exposure for 14 h. The space group and unit-cell parameters were determined from the well resolved diffraction spots (Fig. 2). The data was processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

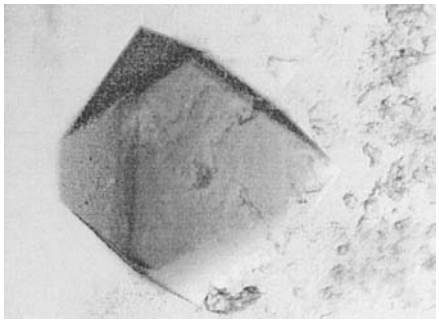

Figure 1
Crystal of agglutinin from the seeds of *A. precatorius*. Only one crystal is present in the drop. The concentration of PEG 8000 was 6.5%.

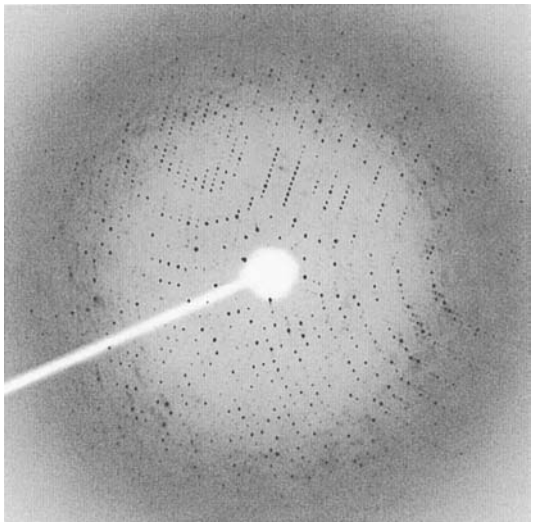

Figure 2
A 1.2° oscillation image of the agglutinin crystal using a Rigaku R-AXIS IV image plate. The resolution at the edge of the image is around 3.0 Å.

The agglutinin crystal belongs to the tetragonal system, with unit-cell parameters $a = b = 141.91$, $c = 105.63$ Å, $V = 2\,127\,224.3$ Å^3, $Z = 8$. A 95% complete data set to 3.45 Å resolution was collected with an $R_{\rm merge}$ of 9.0%. 99 189 reflections were collected, of which 14 718 were unique. The systematic absences, $l = 4n + 1, 2, 3$ for $00l$ reflections and $h = 2n + 1$ for $h00$ reflections, indicate that there are two possible space groups, namely $P4_12_12$ or $P4_32_12$. On the basis of the space-group symmetry, the unit-cell volume and the molecular weight of 134 kDa, a heterotetrameric protein molecule per asymmetric unit was proposed. The V_m value is calculated to be 1.98 Å^3 Da^{-1} assuming one molecule per asymmetric unit, indicating the solvent content to be approximately 38% according to the equation of Matthews (1968). The data were collected twice using different crystals and merged. A summary of the data-collection statistics is given in Table 1.

The sequence of agglutinin has been obtained and published (Liu *et al.*, 2000). To obtain the initial phases for the electron-density map, the molecular-replacement method was applied using the program *X-PLOR* (Brünger, 1992) with the 65 kDa abrin-a as a template (Tahirov *et al.*, 1995); the amino-acid sequence homology between agglutinin and abrin-a is approximately 80%. Intensity data in the resolution range 8.0–4.0 Å were used for the molecular–replacement methods; the vector length is 75 Å. The highest peak from the rotation search was 12.3σ above the mean (the second peak was 8.9σ) and when refined by the Patterson correlation technique resulted in the final Eulerian angles $\theta_1 = 38.4$, $\theta_2 = 37.8$, $\theta_3 = 342.2°$. Subsequently, a 10.8σ solution of the translation function was achieved at unit-cell translations of 0.354, 0.292 and 0.021 along a, b and c, respectively. Calculation of the self-rotation function (Rossmann & Blow, 1962) revealed a large 15.5σ peak at $\varphi = 0$, $\psi = 0$, $\kappa = 180°$, suggesting the presence of a pseudo-twofold non-crystallographic symmetry within the asymmetric unit of crystalline agglutinin; the next peak was at 7.3σ. Finally, assuming two dimers which are related to each other by the pseudo-twofold symmetry (a hetero-tetramer) in the asymmetric unit results in a conventional R factor of 43.2%. Subsequent refinement and analyses is in progress. A search for suitable heavy-atom derivative crystals is also in progress.

Table 1
Data-collection statistics.

Temperature (K)	295
Unit-cell parameters (Å)	$a = b = 141.91$, $c = 105.63$
Space group	$P4_12_12$ or $P4_32_12$
Mosaicity	0.292
Total observations	99189
Unique reflections	14718
Multiplicity	6.74
Resolution range (Å)	
Overall	40.0–3.45
Outermost shell	5.51–3.45
Completeness (%)	
Overall	95.3
Outermost shell	97.0
$I/\sigma(I)$	
Overall	9.2
Outermost shell	3.1
$R_{\rm merge}$ (%)	
Overall	9.0
Outermost shell	39.5

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