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Crystallization and preliminary X-ray study of saporin, a ribosome-inactivating protein from *Saponaria officinalis*

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This paper is dedicated to the memory of Alessià Paglia, prematurely deceased on 10 January 1997.

Abstract

Single crystals of the protein saporin isolated from the seeds of *S. officinalis* have been grown by the vapor-diffusion method using ammonium sulfate as precipitant. The crystals are tetragonal, space group $P4_122$ ($P4_322$), with cell dimensions $a = b = 67.53$ and $c = 119.67$ Å, and diffract to 2.0 Å resolution on a rotating-anode X-ray source. The asymmetric unit contains one molecule, corresponding to a volume of the asymmetric unit per unit mass (V_m) of 2.38 Å^3 Da^{-1}.

1. Introduction

Ribosome-inactivating proteins (RIP's) are plant enzymes that depurinate ribosomal RNA and irreversibly block protein synthesis (Endo & Tsurugi, 1987; Barbieri *et al.*, 1993). They are classified as type-I and type-II RIP's depending on their structure. Type-II RIP's consist of two polypeptide chains (*A* and *B*, about 30 kDa each), the *B* chain being a lectin with cell-binding functions and the *A* chain the catalytic subunit. Type-I RIP's consist instead of the single *A* chain, with the same enzymatic properties as type-II, and lack cell toxicity although they are very active on isolated ribosomes. The single polypeptide of type-I RIP's is usually very basic (p$I \geq 10$). Covalent complexes of monomeric RIP's with monoclonal antibodies (immunotoxins) and other heterologous carriers have been prepared because of their potential as anti-cancer drugs, and are in the trial phase for lymphoma treatment (Dinota *et al.*, 1989; Tonevitsky *et al.*, 1986). Among type-I RIP's, saporin isoform 6, 29 kDa, from the seeds of *S. officinalis* (Caryophyllaceae) is the most widely used because it is easily available and very stable. The chemical modifications introduced into type-I RIP's in order to obtain cross-linking with antibodies may often cause partial or total inactivation of artificial complexes (Lamber *et al.*, 1988). Instead saporin conjugates are in most cases very active, down to picomolar concentrations (Barbieri *et al.*, 1993). Recent studies show that saporin is extremely resistant to denaturation by urea or guanidine (up to 4 *M*) even at a relatively high temperature (up to 294 K). Despite the high coil content (>50%) as determined by circular dichroism spectra, saporin is characterized by an unusual resistance to proteolysis (Santanchè *et al.*, 1997). The remarkable stability of saporin, its capacity to recognize ribosomal proteins and to hydrolyze the *N*-glycosidic bond of one or more adenines from rRNA, and its activity even on nuclear DNA (Barbieri *et al.*, 1994) make this toxin an interesting candidate for structural determination.

The three-dimensional structures of several type-I RIP's have been reported and their overall folds were found to be almost identical (Monzingo *et al.*, 1993; Husain *et al.*, 1994; Hosur *et al.*, 1995). Given that saporin is peculiar among RIP's in stability and activity, it may have a different three-dimensional arrangement of some residues at regions inside or proximal to the active site; therefore, we have undertaken its structural determination by X-ray analysis of single crystals. The preliminary data from crystals of saporin isoform 6, diffracting to 2.0 Å, are reported here.

2. Materials and methods

Saporin was purified from *S. officinalis* seeds following previously described procedures (Stirpe *et al.*, 1983) with some modifications. To improve homogeneity, we fractionated seed extract with acetate-buffered (pH 4.0) ammonium sulfate: the supernatant at 30% saturation of ammonium sulfate was further brought up to 80%, and the pellet suspended and washed with the same solution. Finally, the protein-containing pellet was suspended and extensively dialyzed against 10 m*M* phosphate buffer (pH 7.0). The protein was purified by gel filtration on a Sephadex G100 column in phosphate buffered saline, *e.g.* 10 m*M* phosphate buffer and 0.15 *M* NaCl. The main peak from the G100 column was then purified by ion-exchange chromatography on an S-Resource column (Pharmacia LKB, Uppsala, Sweden), in 10 m*M* phosphate buffer (pH 7.0) and eluted with a linear gradient of NaCl (from 0 to 0.4 *M*). Fractions containing saporin were further purified by gel filtration on a G100 column in 50 m*M* phosphate buffer (pH 7.0) containing 0.5 *M* NaCl. The material was then collected, dialyzed against bi-distilled water, concentrated up to 20 mg ml^{-1} with ultrafiltration and stored in aliquots at 200 K. The protein concentration was measured by the Lowry method.

The conventional hanging-drop method was used to screen crystallization conditions (McPherson, 1990). Various precipitants, buffers of different pH, organic solvents and temperatures were tested. Usually a volume (2 µl) of protein solution at 20 mg ml^{-1} was mixed with an equal volume of reservoir solution and placed on a siliconized cover glass. The cover glass was then inverted and sealed on a reservoir containing 1.0 ml of precipitant solution. In some trials, crystals appeared after a

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Table 1. *Statistical data-collection parameters for saporin as a function of resolution*

Resolution (Å)	R_{merge}†	Multiplicity	Completeness (%)	$I > 3\sigma(I)$ (%)
15.0–4.32	0.050	7.9	95.4	95.4
4.32–3.12	0.103	9.0	100.0	92.8
3.12–2.57	0.128	9.1	99.7	84.9
2.57–2.23	0.195	9.1	99.2	80.3
2.23–2.00	0.294	8.2	96.7	69.0
Overall	0.101	8.7	98.6	82.0

† $R_{merge} = \sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i I_h$, where I_{hi} is the ith observation of the reflection h, while $\langle I_h \rangle$ is the mean intensity of the ith reflection.

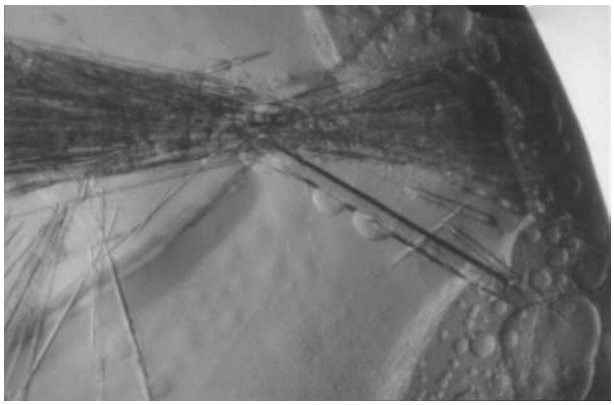

Fig. 1. Photograph of crystals of saporin 6. The largest crystal is 2 mm in its longest dimension.

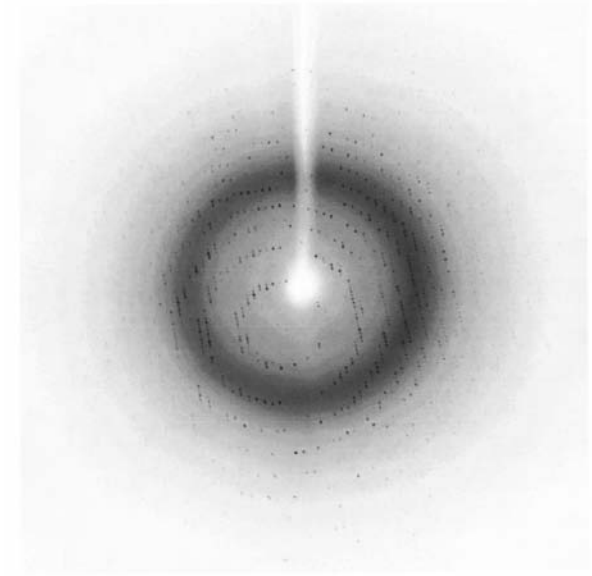

Fig. 2. A typical diffraction pattern of a crystal of saporin 6.

few days. However, in most cases crystals grew as clusters of needles. The best conditions to grow single crystals have been obtained using a reservoir solution containing 2.8 *M* ammonium sulfate and 0.1 *M* sodium citrate at pH = 4.7. The crystallization droplet contained 2 µl of protein solution (20 mg ml^{-1}) mixed with 2 µl of reservoir solution and 2 µl of 10%(*w*/*v*) polyethylene glycol (PEG) 400. The crystallization trials were carried out at a constant temperature of 309 K. The best single crystals grew with maximal dimensions 0.1 × 0.1 × 2.0 mm (Fig. 1).

Crystal were mounted in thin-walled quartz capillaries. A data set to 2.0 Å resolution was collected as 1.8° oscillation frames on an R-AXIS II image-plate detector, mounted on a Rigaku rotating anode, operating at 50 kV and 100 mA, and equipped with a mirror monochromator. Data frames were processed with *DENZO* (Otwinowski, 1993) and scaled with *SCALA* in the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The crystals are stable to X-ray radiation for about 24 h and diffract to 2.0 Å resolution on a rotating-anode X-ray source. The autoindexing procedure of *DENZO* indicated that the crystals belong to the tetragonal crystal system, with unit-cell dimensions $a = b = 67.53$ and $c = 119.67$ Å. Analysis of various data, including a search for systematic absences, showed the data to be consistent with the tetragonal space group $P4_122$ (or $P4_322$) with eight asymmetric units per unit cell. Data processing gave an R_{merge} of 10.1% (based on intensities), the multiplicity is 8.7 and this data set is 98.6% complete to 2.0 Å resolution (96.7% completeness in the resolution interval 2.2–2.0 Å). The percentage of data with $I > 3\sigma(I)$ is 82% (Table 1). Based on the calculated molecular weight of 28 621, assuming one molecule of saporin in the asymmetric unit, the V_m value is calculated to be 2.38 Å^3 Da^{-1}, which falls in the range usually encountered in proteins, *i.e.* the fractional volume occupied by solvent is 48% (Matthews, 1968).

The structure determination by molecular-replacement methods using atomic coordinates of other RIP's (ricin A, pokeweed antiviral protein, and their analogues) deposited in the Protein Data Bank is in progress.

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