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# Crystallization and preliminary crystallographic studies of trichomaglin, a novel ribosome-inactivating protein

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Trichomaglin, a novel ribosome-inactivating protein, has been crystallized in two crystal forms using the hanging-drop vapour-diffusion method. The form *A* and form *B* crystals belong to the orthorhombic space group $P2_12_12_1$ and the hexagonal space group $P6_1$ (or $P6_5$), respectively. X-ray data have been collected to 3.3 and 2.2 Å resolution for the form *A* and *B* crystals, respectively.

## 1. Introduction

Ribosome-inactivating proteins (RIPs) are a family of toxins that inhibit protein synthesis (Barbieri *et al.*, 1993), inactivating ribosomes by hydrolyzing the N—C glycosidic bond of the adenosine residue at the specific site of the large ribosomal subunits. RIPs can be classified into two types. Type I RIPs contain a single polypeptide chain, while type II RIPs consist of two chains (chains *A* and *B*). The molecular weights of type I and type II RIPs are 25–30 and 60 kDa, respectively. Type I RIPs and the *A* chains of type II RIPs possess RNA N-glycosidase activity (Endo & Tsurugi, 1987; Zhang & Liu, 1992), while the *B* chains of type II RIPs assist the *A* chains to enter the cells.

The three-dimensional structures of the type I RIPs such as trichosanthin and $\beta$-momorcharin have been studied by X-ray crystallographic methods in our laboratory (Xia *et al.*, 1993; Yuan *et al.*, 1999). Their overall structures share a common folding pattern and they are similar to those of the *A* chains of type II RIPs such as ricin (Katzin *et al.*, 1991). Some structural differences were observed in loops in the molecular-surface region.

Trichomaglin is a type I RIP with a molecular weight of 24 673 Da isolated from the plant maganlin (*Trichosanthes lepiniana* Naud, Cucurbitaceae family). Trichomaglin and trichosanthin belong to the same genus but different species. We have previously reported the purification, characterization and biological activity of trichomaglin (Chen *et al.*, 1999). The isoelectric point of trichomaglin is 5.8, which is very different from that of trichosanthin (9.4). Trichomaglin can inhibit protein synthesis in rabbit reticulocyte lysate with an $ID_{50}$ of 10.1 n*M*. The amino-acid sequence of the 18 N-terminal residues of trichomaglin shows little sequence homology with trichosanthin for the N-terminal segments (unpublished results). In this paper, we present the crystallization and preliminary crystallographic studies of trichomaglin.

## 2. Crystallization and preliminary crystallographic analysis

The crystallization conditions of trichomaglin were screened using the sparse-matrix sampling method (Jancarik & Kim, 1991) with the hanging-drop vapour-diffusion technique at 293 K. Two crystal forms of trichomaglin have been obtained using PEGs as the precipitant. The orthorhombic form (form *A*) was crystallized in droplets composed of 20 mg ml$^{-1}$ trichomaglin and reservoir solution in a 1:1 ratio; the reservoir solution contained 0.1 *M* Tris–HCl buffer pH 8.5, 28%(*w*/*v*) PEG 4K and 0.2 *M* $Li_2SO_4$. Hexagonal crystals (form *B*) were obtained when 0.15 *M* $KHSO_4$ took the place of $Li_2SO_4$ and the concentration of PEG 4K was decreased to 14%(*w*/*v*). Both forms of the crystals grew to final dimensions of approximately 0.50 × 0.35 × 0.15 mm within a month. The crystals of form *B* diffract better than those of form *A*.

The preliminary crystallographic study was carried out with a precession camera and a Rigaku RU-200 rotating-anode X-ray generator and the space group and the unit-cell parameters were confirmed during the X-ray data collection. The space group and the unit-cell parameters are shown in Table 1. Assuming two and one trichomaglin molecule(s) in the asymmetric unit for form *A* and form *B*, respectively, the volumes per unit protein molecular mass $V_M$ (Matthews, 1968) for the two crystal forms were reasonable, as shown in Table 1.

The X-ray data of both crystal forms of trichomaglin were collected from one single crystal each on a MAR Research 300 imaging-plate detector system. The data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The diffraction data were collected to 3.3 and 2.2 Å resolutions for the form *A* and *B* crystals, respectively. The data-collection statistics are given in Table 1.

**Table 1**
Crystal data of trichomaglin.

| | Form *A* | Form *B* |
|---|---|---|
| Space group | $P2_12_12_1$ | $P6_1$ or $P6_5$ |
| Unit-cell parameters (Å) | | |
| *a* | 49.09 | 94.00 |
| *b* | 68.15 | 94.00 |
| *c* | 153.16 | 57.77 |
| No. of molecules per asymmetric unit | 2 | 1 |
| $V_M$ (Å$^3$ Da$^{-1}$) | 2.60 | 2.99 |
| Resolution (Å) | 3.3 | 2.2 |
| No. of unique reflections | 7732 | 4568 |
| $R_{merge}$† (%) | 0.114 | 0.086 |
| Data completeness (%) | 98.0 | 98.7 |

† $R_{merge} = \sum(|I - \langle I\rangle|)/\sum(I)$.

An attempt was made to determine the crystal structure of trichomaglin by the molecular-replacement method using trichosanthin or $\beta$-momorcharin as the search model, but it failed for both crystal forms of trichomaglin. This fact, along with the very different isoelectric point and lack of sequence homology in the N-terminal segments, suggests that the three-dimensional structure of trichomaglin is probably quite different from that of other type I RIPs such as trichosanthin.

The determination of the primary structure and the crystal structure of trichomaglin are in progress using a gene-sequencing method and the multiple isomorphous replacement method, respectively.

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