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Preliminary crystallographic studies of a protein from *Pachyrrhizus erosus*

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Abstract

Crystals of a protein extracted from the seeds of *Pachyrrhizus erosus* have been obtained by vapor-phase diffusion. The crystal belongs to the space group $P4_12_12$ or $P4_32_12$ with cell parameters $a = b = 62.52$, $c = 147.42$ Å. There is one protein molecule of 33 kDa in an asymmetric unit. A data set at 3.1 Å has been collected on an area detector.

1. Introduction

The ribosome-inactivating proteins (RIP's) have aroused extensive interest owing to their potential use, conjugated with monoclonal antibodies, as immunotoxins to treat cancer (Barbieri & Stirpes, 1982), and owing to the discovery of their anti-HIV activity (Lee-Huang *et al.*, 1990, 1991; McGrath *et al.*, 1989). The RIP's inactivate eukaryotic ribosomes by cleaving a single adenine base from a highly specific site on the 28S rRNA of the 60S ribosome subunit (Endo, Mitsui, Motizule & Tsurgi, 1987). Two types of RIP's, type I and type II, have been reported (Stirpes & Barbieri, 1986). The proteins of type I contain only a single chain, for example, trichosanthin, luffin, momorcharin and pokeweed antiviral protein belong to type I. The proteins of type II contain an *A*-chain and a *B*-chain; ricin and abrin belong to type II.

The *A*-chain of double-chain RIP's, like single-chain RIP's, possesses the RIP property, and *B*-chain of double-chain RIP's is responsible for binding the whole protein molecule to the target cell surface and helping *A*-chain enter the cell membrane.

The crystal structures of ricin (Montfort *et al.*, 1987, Katzin, Collins & Robertus, 1991), trichosanthin (Pan *et al.*, 1993; Gao *et al.*, 1994; Zhou, Fu, Chen, Lin & Pan, 1994), pokeweed antiviral protein (Monzingo, Collins, Ernst, Irvin & Robertus, 1993), and momordin (Husain *et al.*, 1994) have been published. Three-dimensional structure of single-chain RIP's is similar to that of the *A*-chain of double-chain RIP's according to the least-squares superposition of their Cα atom traces (Monzingo *et al.*, 1993; Zhou *et al.*, 1994).

The seed powder of *Pachyrrhizus erosus* has been locally used as an insecticide (Meijer, 1946; Norton, 1943). The toxic principle of the seeds was considered to be a saponin by Nag, Banerjee & Pain (1935). Subsequently Krishnamurti, Sambhy & Seshadri (1970) showed that the toxicity is comparable to that of the rotenone bearing roots of *Derris* and *Lonchocarpus*. From the seeds we have isolated and purified two proteins with molecular mass of 33 and 26 kDa, respectively, which may be members of an RIP's class based on the similar molecular mass of type I RIP's. Research on the biological functions of these proteins is under way. The isolation and purification of these proteins, as well as their biological activities, will be described elsewhere.

2. Experiment

For the protein of molecular mass 33 kDa the conventional hanging-droplet method was used to screen various buffers of different pH and precipitant, as suggested by Jancarik & Kim (Jancarik & Kim, 1991). The crystals were grown in 4–7 d at room temperature in several conditions containing 2-propanol. A condition of crystallization has been found to be in an acetic acid buffer solution. Generally, 6 µl of buffered protein solution at a concentration of 20 mg ml^{-1} was placed on a silanized cover-glass, the cover-glass was then put over a well that contained about 0.8 ml of solution with 100 m*M* acetic acid, 20%(*v*/*v*) 2-propanol, and 200 m*M* calcium chloride at pH = 4.6. The droplet consists of the equal volume of protein solution and the solution from the well. The crystals grew in the octagonal plate form with twining in the direction of the *c* axis within 10 d. Dioxane of 3%(*v*/*v*) has been used to overcome the crystal twinning.

A crystal suitable for X-ray diffraction, with size of 0.5 × 0.25 × 0.1 mm, was mounted in a thin-walled quartz capillary and set on a precession camera to take a photograph, and then on an area detector. The crystallographic parameters were determined as $a = b = 62.52$, $c = 147.42$ Å in tetragonal form. The systematic absences indicate that the space group is $P4_12_12$ or $P4_32_12$. Assuming that there is one molecule in the asymmetric unit, the V_m value (Matthews, 1968) is calculated to be 2.18 Å^3 Da^{-1} which means that the solvent content is approximately 44%(*v*/*v*). A data set at 3.1 Å resolution has been collected on an area detector (Siemens X-200 B) in Beijing.

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