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Crystallization and preliminary crystallographic studies of pink color chromoprotein from *Pleurotus salmoneostramineus* L. Vass.

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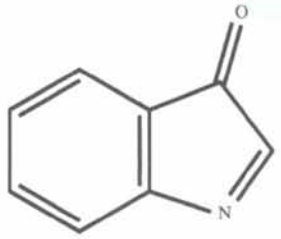
Abstract

A chromoprotein from *Pleurotus salmoneostramineus* L. Vass. has been purified and crystallized. The needle-shaped crystal has monoclinic space group $C2$ with the cell dimensions of $a = 118.5$, $b = 59.7$, $c = 31.8$ Å and $\beta = 114°$. The crystal diffracts to 1.8 Å resolution with a synchrotron radiation X-ray source.

1. Introduction

Pleurotus salmoneostramineus L. Vass., a type of mushroom found in Siberia, Japan and New Guinea, is known for its beautiful pink color. This mushroom includes a pink chromoprotein with an absorption spectrum maximum at 496 nm. This protein has a pigment molecule, 3*H*-indol-3-one (I) which occupies a part of the active site (Takekuma, Takekuma, Matsubara, Inaba & Yoshida, 1994) and has three kinds of metals, Zn, Fe, and Cu. 3*H*-indol-3-one has an absorption maximum at 456 nm in methanol and a bathochromic shift occurs in the chromoprotein. Takekuma *et al.* have reported that the protein produces oxygen molecules from water on light irradiation. This phenomenon implies that



(I)

this mushroom has a photosynthetic function in which the chromoprotein plays an important role (Takekuma *et al.*, 1994). The aim of the present study is to reveal the structure–function relationship of the chromoprotein with the novel photosynthetic mechanism.

2. Purification and crystallization

Chromoprotein was extracted by soaking the mushrooms in water. Crude chromoprotein was freeze-dried and stored at 195 K. The protein (0.5 g) was dissolved in 1 ml of Tris–HCl buffer (pH 7.0), then applied onto a Sephacryl S-300HR column (Pharmacia, 3 × 80 cm, pre-equilibrated in Tris–HCl buffer, pH 7.0). The fractions containing the chromoprotein were collected and dialyzed for 12 h against glycine–NaOH buffer (pH 10.0). It was loaded on DEAE–Sepharose column (2 × 30 cm) equilibrated in glycine–NaOH buffer (pH 10.0) and then washed with 20 m*M* NaCl solution in the elution buffer. The purified protein was dialyzed against water and concentrated up to 10 A cm^{-1} at 496 nm.

Single crystals were obtained by the vapor-diffusion method. The reservoir consists of 50 m*M* buffer solution (Tris–HCl, pH 9.0) containing 21%(*w*/*v*) polyethylene glycol 4600 (Sigma) and 200 m*M* sodium formate. The protein drop was prepared by mixing 3µl of protein solution with 3µl of reservoir solution. Single red-colored crystals appeared as prismatic needles within one week (Fig. 1).

3. X-ray analysis

Preliminary X-ray experiments were carried out on a Rigaku R-AXIS IIC imaging-plate detector system equipped with a Rigaku RU-300 rotating-anode X-ray generator (fine-focused Cu $K\alpha$, operating at 40 kV and 100 mA). The Laue symmetry and cell dimensions were determined by the *PROCESS* program package (Higashi, 1989; Sato *et al.*, 1992) and systematic absences were confirmed by pseudo-precession pictures using the program *HKLPLOT* (Eleanor Dodson, unpublished data; Collaborative Computational Project, Number 4, 1994). The crystals belong to monoclinic space group $C2$ with the unit-cell dimensions $a = 118.5$, $b = 59.7$, $c = 31.8$ Å and $\beta = 114°$. Supposing one molecule per asymmetric unit, V_m value is calculated to be 2.1 Å^3 Da^{-1} (Matthews, 1968). Diffraction was found on imaging plates up to 2.5 Å resolution.

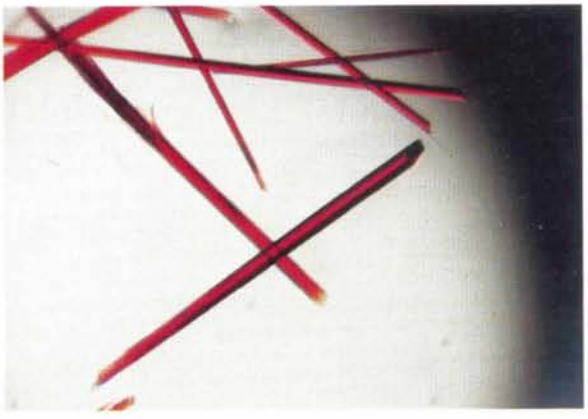


Fig. 1. Crystals of chromoprotein from *Pleurotus salmoneostramineus* L. Vass. The biggest crystal is approximately 0.5 × 0.1 × 0.07 mm.

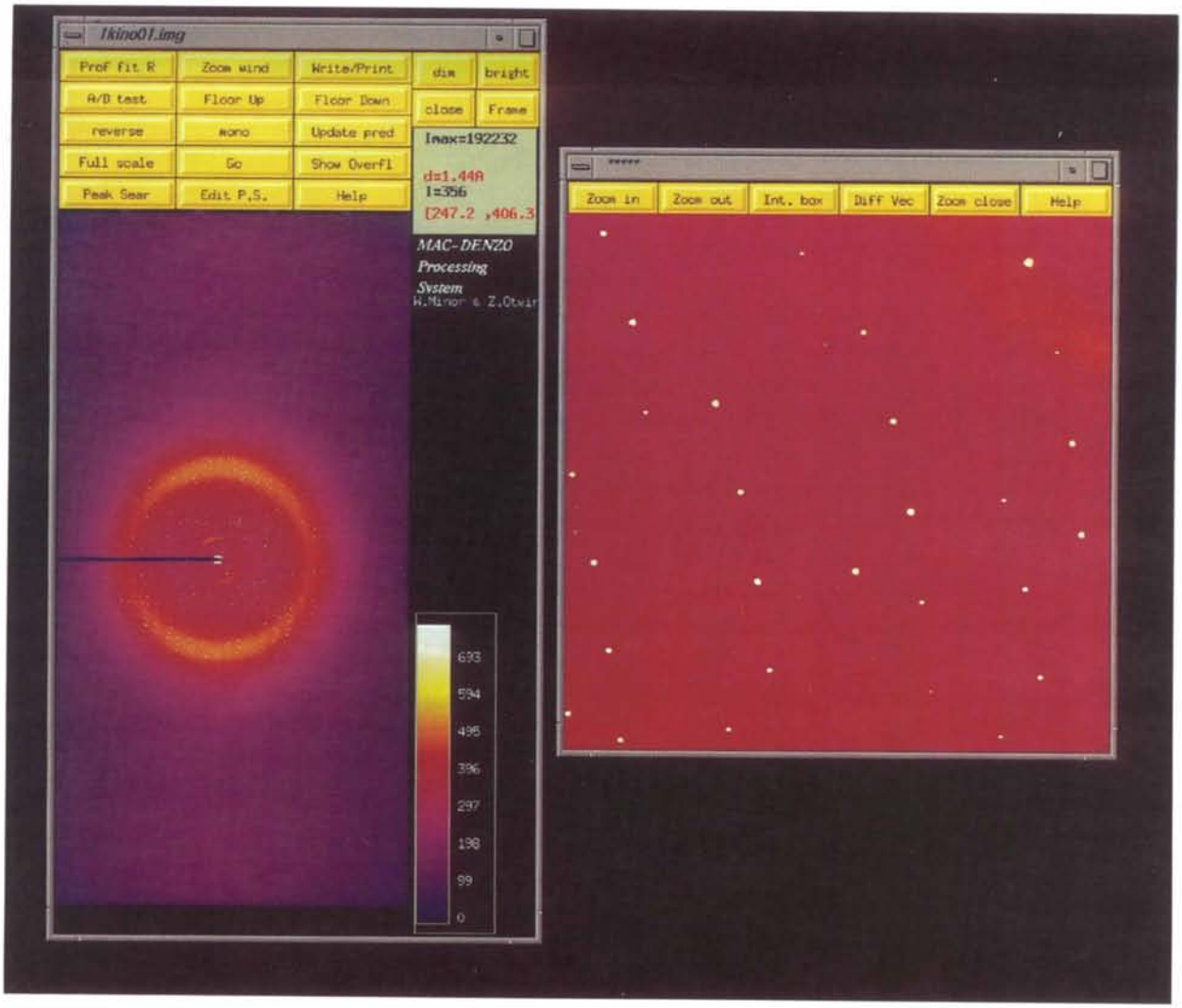


Fig. 2. A Weissenberg diffraction pattern from a chromoprotein crystal (rotation axis *b*). This image was obtained with a Weissenberg camera for macromolecules (Sakabe, 1983; cassette distance and radius are 430 mm) equipped with an imaging plate (40 × 80 cm).

Diffraction intensity data were collected by using synchrotron radiation (BL-18B beamline, Photon Factory, KEK, Japan). The Weissenberg camera for macromolecules (Sakabe, 1983) and large imaging plates (40 × 80 cm) were used for data collection. The crystal diffracts up to 1.8 Å resolution and 15 frames with a rotation angle of 12.5° for each frame were stored (Fig. 2). During the data collection no obvious radiation damage was detected. Data processing was carried out by the programs *DENZO* and *SCALEPACK* (Otwinowski, 1993). The combined set gave 71 241 reflections to 1.8 Å resolution in total, which were reduced 18 198 unique reflections with an $R_{\rm merge}$ of 4.8% ($R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$) and the completeness of 85.7% (61.4% for 1.80–1.86 Å).

A search for heavy-atom derivatives is currently under way for structure determination by isomorphous replacement method.

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