

X-ray Crystallographic Study of Pink Color Chromoprotein from *Pleurotus Salmoneostramineus* L. Vass.

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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, 3H-indol-3-one which occupies a part of the active site (Takekuma, 1994) and has three kinds of metals, Zn, Fe, and Cu. 3H-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 produced oxygen molecules from water on light irradiation. This phenomenon implies that this mushroom has a photosynthetic function in which the chromoprotein plays an important role (Takekuma et al., 1994). We had already reported the crystallization method. The needle-shaped crystal has monoclinic space group *C*2 with the cell dimensions of $a = 118.5$, $b = 59.7$, $c = 31.8$ Å and $\beta = 114^\circ$. We had obtained the diffraction data up to 1.8 Å resolution with an R_{merge} of 4.8% and the completeness of 85.7% (61.4% for 1.80 - 1.86 Å). Structural analysis of chromoprotein is attempted by the isomorphous replacement method with anomalous scattering. We have

already the partial structure of chromoprotein, however, it is difficult to determine the structure of the active site with current electron density map. There is no information of amino acid sequence for the protein. To collect a higher resolutional data we used synchrotron radiation at BL-41XU station produced by the storage ring in Spring-8. The camera for macromolecules and large imaging plates were used for data collection. The intensity data was collected at 0.708 Å monochromatized by an Si(111) monochromator system. Diffraction patterns were recorded on a Fuji Imaging Plate and the read-out IP data was carried out by a RIGAKU Large-IP reader. The first image have quite clear spots of the diffraction, however, the crystals might be damaged by the strong X-ray. Moreover, we couldn't prepare the crystals with high quality for the specified schedule. We used also the cryo-system at beam line 41-XU, however all images have ice-rings in spite of many trials. Finally, an R_{merge} value for the data collected was 10.3 % for 6,545 reflections up to 2.5 Å resolution data obtained at room temperature (completeness 85.1%).