

**INHIBITOR BINDING OF NITRILE HYDRATASE FROM
RHODOCOCOCCUS SP. N771 IN THE PHOTOACTIVATION PROCESS**

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Nitrile Hydratase (NHase) from *Rhodococcus sp.* N771 contains a mononuclear non-heme iron center. NHase is inactivated by nitrosylation. The nitric monoxide molecule on the iron center is dissociated by photo-irradiation, possibly replaced with a water molecule in the active state. Sulfur atoms of α Cys112 and α Cys114, coordinating to the iron center, are post-translationally modified to cysteine-sulfinic (α Cys112-SO₂H) and cysteine-sulfenic acid (α Cys114-SOH), respectively (Nat. Struct. Biol., 5, 347-351, 1998). To study the photoactivation process and the reaction mechanism of NHase, we prepared the crystals of the complex between inactive NHase and an irreversible inhibitor, cyclohexyl isocyanide by soaking experiments and investigated the structural changes induced by photo-irradiation. The crystals were irradiated with a normal fluorescent lamp (14 mW/cm²) for 0hr, 0.75hr, 1.0hr, 1.5hr, and 2.0hr at 4°C and stored in the liquid nitrogen. X-ray diffraction data sets for the crystals were collected in the light using BL44B2 at SPring-8. Crystal structures were refined using CNS and SHELX-97 at 1.5, 1.6, 1.7, 1.2, and 1.7 Å resolutions to R-factors of 15.5, 14.9, 15.5, 15.5, and 14.8%, respectively. Upon photoactivation, the isocyanide group of the inhibitor turned to the opposite direction and bound directly to the iron. Surprisingly, the electron density for the O δ of α Cys114-SOH gradually decreased and disappeared at 1.0hr photo-irradiation, but appeared again in the crystals further irradiated. O δ of α Cys114-SOH might be replaced with an oxygen atom of water molecule during inhibitor binding to NHase.

**Keywords: CYSTEINE SULFENIC ACID PHOTOACTIVATION
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**CRYSTAL STRUCTURES OF REACTION INTERMEDIATES OF AN
EXTRADIOL-CLEAVING CATECHOLIC DIOXYGENASE**

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BphC derived from *Pseudomonas sp.* strain KKS102 is an extradiol-cleaving catecholic dioxygenase. This enzyme contains a non-heme iron (Fe(II)) and plays an important role in degrading biphenyl/PCBs (polychlorinated biphenyls) in the microbe. In order to elucidate the catalytic mechanism of the enzyme, crystal structures of the substrate free form, the BphC-substrate (ES) complex, and the BphC-substrate-NO (ES-NO) complex were determined under anaerobic conditions. These crystal structures revealed the followings. (a) The substrate directly coordinates to the Fe ion as a monoanionic form. (b) Upon substrate binding, His194 makes a conformational change, forming a strong hydrogen bond with hydroxyl group of the substrate. This hydrogen bond seems to be required to deprotonate the hydroxyl group. (c) The NO molecule directly coordinates to the Fe ion. The binding site of the NO molecule, which is highly likely to be the binding site of dioxygen, is the vacant site of the octahedral coordination sphere of the ES complex. The cavity that accommodates the NO molecule is lined by hydrophobic residues. On the basis of these findings, we propose a catalytic mechanism of the extradiol-cleaving catecholic dioxygenase in which His194 seems to play two distinct roles. At the early stage of the catalytic reaction, His194 appears to act as a catalytic base, which likely deprotonates the hydroxyl group of the substrate. At a later stage, the protonated His194 seems to stabilize a negative charge on the oxygen molecule located in the hydrophobic oxygen-binding cavity

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**X-RAY ANALYSIS OF MntC, A Mn-TRANSPORTER PERIPLASMIC
PROTEIN IN THE SYNECHOCYSTIS SP. PCC 6803**

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In the photosynthetic cyanobacterium, *Synechocystis sp.* PCC 6803, high affinity manganese import is carried out by an ABC (ATP-binding cassette) transporter. It consists of an ATP-binding protein (MntA), an integral membrane protein (MntB) with eight putative transmembrane regions and a periplasmic substrate-binding protein (MntC), which protein is a membrane-anchored lipoprotein. An unanchored soluble form of MntC has been shown to be fully active in manganese transport both in vivo and in vitro. Knowledge of the three-dimensional structure of MntC will help not only to understand how manganese is bound and delivered into the cell but also may elucidate some of the steps necessary for manganese mobilization into cyanobacterial Photosystem II. Overexpressed recombinant MntC protein was found in inclusion bodies. Unfolding was performed using saturated urea solution and refolding by one step dilution. Purified protein was obtained by ammonium sulfate precipitation and anion exchange HPLC. MntC protein eluted in multiple fractions. The calculated molecular weight of the purified protein by size exclusion chromatography HPLC indicated that the MntC formed monomers, dimers and tetramers. Crystals of MntC-dimer were grown in the presence of 10%-15% PEG 4000 and 0.05 - 0.1 M ZnAc in 0.1 M cacodylic acid (pH 6.5). The crystals diffracted to a maximum resolution of 2.6 Å. Analysis of the diffraction pattern using either DENZO or Mosfilm indicated that the MntC crystals belong to a hexagonal space group with unit cell dimensions of 126 Å x 126 Å x 88 Å. A number of native data sets, 100% complete to 3.0 Å were collected. Structure determination by Multiple Isomorphous Replacement (MIR) is being performed at present.

**Keywords: MANGANESE TRANSPORT, CYANOBACTERIA,
PHOTOSYNTHESIS**

**CRYSTAL STRUCTURE OF WATER-SOLUBLE CHLOROPHYLL
PROTEIN FROM RAPHANUS SATIVUS**

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A water-soluble chlorophyll protein (WSCP) was found in *Chenopodium album* first in 1963. Subsequent studies have shown that some other plants in *Polygonaceae*, *Chenopodiaceae*, *Amaranthaceae* and *Brassicaceae* also contain WSCPs. These WSCPs can be categorized into two classes depending on their photoconvertibility. The WSCP from *C. album* shows drastic change in the absorption spectrum and pI with light exposure[1]. The WSCP from *Raphanus sativus* shows no photoconvertibility. Crystals of the WSCP belong to space group C222 with unit-cell dimensions of a = 98.1, b = 184.2, c = 91.6 Å. The three-dimensional structure was solved by molecular replacement using WSCP from *Lepidium virginicum* as a search model. The molecule consists of four subunits and contains four chlorophylls (Chls). Two Chls form a sandwich structure in the molecular core. The subunits have a beta-trefoil structure which consists of a 6-stranded β -barrel and 3 β -sheets. So far the structures of WSCPs from *L. virginicum* and *Brassica oleracea* have been determined. Comparison of the three structures shows that dimers in which Chls form a sandwich structure have almost the same structures, whereas arrangement of the dimers vary among these three molecules. The dihedral angles between the Chl plane of one dimer and that of another dimer in the WSCPs from *R. sativus*, *L. virginicum* and *B. oleracea* are 58.89°, 36.47° and 83.52°, respectively.

References

[1] Satoh, H., Uchida, A., Nakayama, K. and Okada, M. (2001) Plant Cell Physiol. 42, 906-911

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