

## Poster Presentation

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### *Crystal structure of pyridoxine 4-oxidase from Mesorhizobium loti*

A. Mugo<sup>1</sup>, J. Kobayashi<sup>2</sup>, T. Yamasaki<sup>3</sup>, B. Mikami<sup>2</sup>, Y. Yoshikane<sup>3</sup>, T. Yagi<sup>3</sup>, K. Ohnishi<sup>1</sup>

<sup>1</sup>Kochi University, Research Institute of Molecular Genetics, Kochi, Japan, <sup>2</sup>Kyoto University, Division of Applied Life Sciences, Gokasho, Japan, <sup>3</sup>Kochi University, Graduate School of Integral Arts and Science, Kochi, Japan

*Mesorhizobium loti* MAFF303099, a nitrogen-fixing symbiotic bacterium, harbors degradation pathway I for pyridoxine (PN); a free form of vitamin B6. Pyridoxine 4-oxidase (PNOX), a monomeric glucose-methanol-choline (GMC) oxidoreductase family enzyme, is the first enzyme in the pathway. It catalyzes FAD-dependent oxidation of pyridoxine (PN) into pyridoxal. PNOX with a C-terminal His6 tag was overexpressed in *E. coli* JM109 cells and purified with a Ni-NTA agarose column and a QA52 column. The tertiary structures of PNOX and a complex of PNOX with pyridoxamine (PM), which is a substrate analog, were determined at 2.2 Å and at 2.1 Å resolutions, respectively. The overall structure consisted of FAD-binding and substrate-binding domains. The FAD interacts with the PNOX protein through a network of hydrogen bonds, which are mainly found in the ribose and pyrophosphate moieties of the FAD molecule. The surface structure of PNOX molecule showed that it had an opening socket for access of substrates. The opening was followed by a tunnel that was linked to the active site cavity. In the active site, His460, His462, and Pro504 were located on the re-face of the isoalloxazine ring of FAD. PM binds to the active site through several hydrogen bonds. The side chains of His462 and His460 are located at 2.7 and 3.1 Å from the N4' atom of PM. The activities of H460A and H462A mutant PNOXs were very low, and H460A/H462A double mutant PNOX exhibited no activity. His462 may act as a general base for abstraction of a proton from the 4'-hydroxyl of PN. His460 may play a role in the binding and positioning of PN. The C4' atom in PM is located at 3.2 Å, and the hydride ion from the C4' atom may be transferred to the N5 atom of the isoalloxazine ring. The comparison of active site residues in GMC oxidoreductase family shows that Pro504 in PNOX corresponds to Asn or His of the conserved His-Asn or His-His pair in other GMC oxidoreductases.

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The cartoon view of PNOX-PM complex structure. The substrate-binding domain is colored red; the FAD-binding domain green; FAD orange and PM blue

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