We crystallized aldehyde reductase from *S. salmonicolor* for X-ray structure analysis. There is a class of monomeric NADH-dependent oxidoreductases with molecular masses about 35,000 which is called the aldo-keto reductase superfamily. Aldehyde reductase of *Sporobolomyces salmonicolor* well catalyzes not only the NADH-dependent reduction of p-nitrobenzaldehyde and pyridine-3-aldehyde, which were typical substrates for mammalian aldehyde and aldo reductases, but also that of prochiral carbon compounds such as 4-chloro-3-oxobutanoate esters. Since the later products are optically active alcohols, the enzyme could recognize the stereoposition of these substrates. This stereoselectivity might be dependent on the tertiary structure of the enzyme, therefore elucidation of the tertiary structure of aldehyde reductase is significant for elucidation of mechanism controlling stereoselectivity.

Hexagonal crystals were obtained from ammonium sulfate solution by vapor diffusion (M. Kataoka, et al., Acta Cryst., in press). The space group is P6₁₂₂ or P6₁₅,2 with unit cell dimensions of *a*=72.2 Å, *c*=320 Å. Assuming two molecules are in the asymmetric unit, *V*=1.7 Å³/Da. Intensity data were collected on a Weissenberg camera with synchrotron radiation at BL-6A at the Photon Factory, KEK, Japan. Intensities were processed and scaled up to 2.2 Å resolution. The Rmerge is 5.9% for 18535 independent on the native crystal. The structure analysis is in progress.

We crystallized and characterized the major NAD(P)/H·FMN oxidoreductase (flavin reductase) of a bioluminescent bacterium *V. fischeri*. We crystallized and characterized the major NAD(P)/H·FMN oxidoreductase (flavin reductase) of a bioluminescent bacterium *V. fischeri*. We crystallized and characterized the major NAD(P)/H·FMN oxidoreductase (flavin reductase) of a bioluminescent bacterium *V. fischeri*.

The enzyme catalyzes the reduction of FMN to FMNH₂ using NAD(P)/H as an electron donor and supplies the FMNH₂ to bacterial luciferase. The enzyme was purified from an expression culture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan, 3Department of Biotechnology, Faculty of Engineering, Tottori University, Tottori 680, Japan

We crystallized and characterized the major NAD(P)/H·FMN oxidoreductase (flavin reductase) of a bioluminescent bacterium *V. fischeri*. While the enzyme could reduce cofactor FMN, then NAD+ leaves from the enzyme. Next, a substrate FMN binds to the enzyme and gives 2 electrons from the reduced cofactor FMN.

Crystals were obtained using PEG 4000 as a precipitant. Precession photographs revealed that the crystals belong to monoclinic, space group C₂₁ with cell parameters of *a*=101.6, *b*=63.3, *c*=74.4 Å, and β=100.0° and have a dimer in an asymmetric unit. We collected the native intensity data using synchrotron radiation in the Photon Factory at National Laboratory for High Energy Physics. The data sets had the statistics of 79.9% completeness with 25,163 independent reflections (2.2 Å resolution). The data of the crystals soaked in solutions containing heavy atom compounds indicated a good isomorphous derivative of a mercury compound, EMTS. We also observed anomalous scattering data of the derivative, and calculated isomorphous phase with the program MLPHARE of CCP4. Figure of merits and phasing power for centric reflections at 2.7 Å resolution were 0.75 and 1.35, respectively. To improve the phase, we are searching another good derivative.

D-amino acid oxidase [D-amino acid: O₂ oxidoreductase (deaminating), EC 1.4.3.3 (DAO)] was discovered more than 60 years ago as the first mammalian flavoprotein. DAO possesses one molecule of FAD noncovalently bound to each subunit of molecular mass of 39,000 Da with the known primary structure and nucleotide sequence of the cDNA. Numerous chemical modifications studies as well as mutagenesis studies have provided clues for the catalytically important residues. However, neither these primary structural information nor modification/mutation studies suffice to understand the catalytic events of DAO at the molecular or submolecular level. In order to elucidate catalytic events of DAO, we started to X-ray crystallographic study.

Crystallization of DAO was performed by hanging-drop method and crystals with good quality for X-ray crystallography were obtained. The crystals so obtained diffracted to 2.5 Å with a conventional X-ray source. The crystals belong to the orthorhombic space group *P₂₁2₁2₁* with unit cell dimensions of *a*=110.3, *b*=92.9, *c*=71.6 Å. The anomalous difference map of 2.35 Å/Da indicates two subunits related by a twofold noncrystallographic axis in the asymmetric unit. Two heavy atom derivatives have been identified. Data collection was performed on a Rigaku RAXIS IIc, and on a synchrotron beam line BL6A of the Photon Factory.

Crystallization studies of LMO have been presented. The structure determination will be presented.

**CRYSTALLOGRAPHIC STUDY OF D-AMINO ACID OXIDASE.** Hitashi Mizutani*, Ikuko Miyahara*, Ken Hirotsu*, Chiiki Setoyama**, Yasuo Nishina**, Kyosshi Shiga**, and Retsu Miura** Department of Chemistry, Faculty of Science, Osaka City University, Departments of Biochemistry and Physiology, Kuma-**n**o University School of Medicine.

D-amino acid oxidase [D-amino acid: O₂ oxidoreductase (deaminating), EC 1.4.3.3 (DAO)] was discovered more than 60 years ago as the first mammalian flavoprotein. DAO possesses one molecule of FAD noncovalently bound to each subunit of molecular mass of 39,000 Da with the known primary structure and nucleotide sequence of the cDNA. Numerous chemical modifications studies as well as mutagenesis studies have provided clues for the catalytically important residues. However, neither these primary structural information nor modification/mutation studies suffice to understand the catalytic events of DAO at the molecular or submolecular level. In order to elucidate catalytic events of DAO, we started to X-ray crystallographic study.