PS04.01.83 X-RAY STRUCTURE ANALYSIS OF ALDEHYDE REDUCTASE FROM A RED YEAST. A. Kita[1], M. Kataoka[2], K. Yamamoto[2], S. Shimizu[2], K. Kita[3], T. Hashimoto[3], H. Yamane[3], K. Miki[1], [1]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan, [2]Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan, [3]Department of Biotechnology, Faculty of Engineering, Tottori University, Tottori 680, Japan

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 aldose reductases, but also that of prochiral carbonyl 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 $P6_122$ or $P6_522$ with unit cell dimensions of a=b=72.2Å, c=320Å. Assuming two molecules are in the asymmetric unit, V_m is calculated to be 1.7Å^3/Da. Intensity data were collected on a Weissenberg camera with synchrotron radiation at BL-6A$_2$ at the Photon Factory, KEK, Japan. Intensities were processed and scaled up to 2.2Å resolution. The Rmerge is 5.9% for 18535 independents on the native crystal. The structure analysis is in progress.

PS04.01.84 CRYSTALLIZATION AND CRYSTALLOGRAPHY OF THE MAJOR FLAVIN REDUCTASE OF *Vibrio fischeri*. Hideaki Koike[1], Hiroshi Sasaki[1], Shuhei Zenno[2], Kaoru Saigo[2], Michael E. P. Murphy[3], Elinor T. Adman[3], Masaru Tanokura[1], [1]Biotechnology Research Center, [2]Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan, 3Department of Biological Structure, Box 357420, School of Medicine, University of Washington, Seattle, WA 98195-7420, USA

We crystallized and characterized the major NAD(P)H:FMN oxidoreductase (flavin reductase) of a bioluminescent bacterium *V. Fischeri*. It diffracted X-ray to further than 2.2 Å. We have found a good derivative of mercury and are now searching other derivatives to get better isomorphous phasing.

The enzyme catalyzes the reduction of FMN to $FMNH_2$ using NAD(P)H as an electron donor and supplies the $FMNH_2$ to bacterial luciferase. The enzyme was purified from an expression system of *E. coli* and characterized on the biochemical properties. The enzyme works as a dimer and has FMN as a cofactor. From the steady state kinetics and anaerobic reductive titration by dithionite and NADH, we have proposed the reaction mechanism of the enzyme as follows: 2 electrons flow from NADH to the cofactor FMN, then NAD+ leaves from the enzyme. Next, a substrate FMN binds to the enzyme and is given 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 C_2, and cell parameters were 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 23,163 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.

PS04.01.85 CRYSTALLOGRAPHIC STUDY OF D-AMINO ACID OXDASE. Hisashi Mizutani*, Ikuko Miyahara*, Ken Hirotsu*, Chiaki Setoyama**, Yasuzo Nishina***, Kiyoshi Shiga***, and Retsu Miura** Department of Chemistry, Faculty of Science, Osaka City University*, Departments of Biochemistry** and Physiology***, Kuma~noto University School of Medicine

D-amino acid oxdase [D-amino acid: O_2 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 2.5Å with a conventional X-ray source. The crystals belong to the orthorhombic space group $P2_12_12_1$ with unit cell dimensions of a=110.3, b=92.9, c=71.6 Å. A V_m value of 2.35 Å^3/Da indicates two subunits related by a two-fold 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 screenless Weissenberg camera, using a synchrotron radiation at a beam line BL6A of the Photon Factory.

PS04.01.86 CRYSTALLOGRAPHIC STUDES OF LACTATE MONOOXYGENASE (LMO) AND OLD YELLOW ENZYME2 (OYE2). Zhan Deng, P. Andrew Karplus, Section of Biochemistry, Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, Vincent Massey, Department of Biological Chemistry, Medical School, University of Michigan, Ann Arbor, MI48109

The flavoprotein L-lactate 2-monooxygenase (LMO) catalyzes the oxidative decarboxylation of L-lactate to acetate, carbon dioxide and water. Although LMO is closely related to glycollate oxidase (GOX) and flavocytochrome b2 (FCB), it has notably different kinetic properties compared to GOX and FCB. In particular, GOX and FCB have keto acids as their final oxidative products, whereas in LMO, the intermediate pyruvate is tightly bound to the enzyme and is subsequently decarboxylated. Crystallographic study of LMO will provide a good opportunity to see how subtle structural change can lead to large change in function.

Crystals of LMO from *Mycobacterium smegmatis* have been grown in a primitive tetragonal space group with a=b=143Å, c=269Å. A 2.6Å data set has just been collected from a frozen LMO crystal at CHESS. We plan to solve the structure by molecular replacement method using GOX as a search model, and gain insights into the structural cause of their functional differences. An update of the structure determination will be presented.