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.

Old yellow enzyme (OYE) (E.C. 1.6.99.1) was the first identified flavoprotein and has been used as a model protein in the studies of flavoprotein and flavin chemistry. However its physiological function still remains elusive. Recent discoveries that OYE interacts with actin protein and deletion of OYE genes in *S. cerevisiae* leads to perturbed actin cytoskeleton indicate that OYE plays an important role in yeast. The structure of old yellow enzyme from brewer's yeast (OYE 1) was solved to 2.0Å resolution, showing a TIM-barrel domain structure.

We have grown crystals of OYE2, an OYE isoform from *S. Cerevisiae*. The crystals belong to space group $P2_12_12_1$ (a=70Å, b=96Å, c= 151Å) and diffract to 2.5Å resolution. The structure determination is in progress by molecular replacement and we plan to report a comparison between the structures of OYE1 and OYE2 isoforms.

PS04.01.87 STRUCTURE OF THE FLAVOENZYME D-AMINO ACID OXIDASE. M. Bolognesi[1,3], A. Mattevi[1], M.A. Vanoni[2], F. Todone[1], B. Curti[2], [1]Dip. Geneticae Microbiol. Univ.Pavia, via Abbiategrasso 207, 27100 Pavia, [2]Dip. Biochimica e Fisiologia Gen. Universita' di Milano, via Celoria 26, 20133 Milano; [3]Dip.Fisica Centro Biotec.Avanzate, Univ.Genova, L.go R.Benzi 10, 16132 Genova, Italy

D-amino acid oxidase is the prototype of the oxidase class of FAD-dependent enzymes. The protein catalyses the oxidative degradation of D-amino acids to the corresponding keto acids with the release of ammonia and hydrogen peroxide. The enzyme displays a broad substrate specificity and is capable of oxidising several D-amino acids. We have determined the three-dimensional crystal structure of pig kidney D-amino acid oxidase by multiple isomorphous replacement and eight-fold averaging.

The overall structure of D-amino acid oxidase encompasses two well characterised domains, which define at their interface the flavin ring binding site. The position of the competitive inhibitor benzoate allows the identification of the residues likely to take part in catalysis. Inspection of the active site reveals that there are no residues properly positioned to act as the active site base required for the carboanion mechanism, which has been postulated by most investigators. On the contrary, the crystallographic analysis suggests that the reaction proceeds by direct hydride transfer from the substrate Ca atom to the flavin N5 atom. The active site of D-amino acid oxidase closely resembles that of flavocytochrome b2, a structurally unrelated FMN-dependent enzyme. The catalytic groups of the two enzymes are well superimposable once the mirror-image of flavocytochrome b2 is generated with respect to the flavin. This fact finds a precise explanation in the opposite stereospecificity of the two proteins, thus suggesting that flavocytochrome b2 and D-amino acid oxidase represent a striking example of mirror image convergent evolution.

PS04.01.88 THE CATALYTIC MECHANISM OF SUPEROXIDE DISMUTASE BASED ON CRYSTALLOGRAPHIC STUDIES OF THE REDUCED ENZYME AND INHIBITOR COMPLEXES. Keith S. Wilson[1], Marta Ferraroni[2], Pier Luigi Orioli[3], Wojciech R. Rypniewski[1] & Stefano Mangani[2]. [1]European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany; [2]Department of Chemistry, University of Siena, Pian dei Mantellini 44, I-53100 Siena, Italy; [3]Department of Chemistry, University of Florence, via G. Capponi 7, I-50121 Firence, Italy.

A new crystal form of bovine superoxide dismutase is studied in which the copper ion is reduced to Cu(I), as in the course of the dismutation reaction. The structure has an unusually high solvent content, with V_M = 4.5 Å/dal, from which the solvent content can be estimated at 73%. Crystal structures have been solved of the unliganded enzyme and in complex with inhibitors, azide and thiocyanide. The results are used in the study of the catalytic mechanism of SOD. The new crystal form of reduced SOD confirms our earlier observation that the imidazole of His61 is not protonated upon reduction of copper, that the coordination of the metal ions is maintained and that the protons necessary for the reaction can be efficiently provided directly from the solvent. In addition, the crystal structure of reduced SOD and its complexes with anions are compared with the structure of the analogous adducts of oxidised SOD. The azide complex with reduced SOD and that with oxidised SOD fit with the mechanism proposed by Osman & Basch in which a stable complex between Cu(II) SOD and superoxide is formed and an outersphere electron transfer occurs in the first part of the cycle. Once Cu is reduced, it gives back one electron to the bound superoxide which is released as hydroperoxide anion. Different results obtained by NMR and other spectroscopic techniques suggest that the detailed reaction mechanism could differ depending on conditions.

PS04.01.89 THE THREE DIMENSIONAL STRUCTURE OF MOUSE NAD(P)H: QUINONE REDUCTASE EXPRESSED IN E. COLI. Mario A. Bianchet[1], Margarita Faig[1], Shiuan Chen[2], Paul Talalay[3] and L. Mario Amzel[1], Dept. of Biophysics & Biophysical Chemistry, [1]Johns Hopkins School of Medicine, Baltimore, MD, 21205, Division of Immunology, [2]Beckman Research Institute of the City of Hope, Duarte, CA 91010, [3]Dept. of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Quinone Reductase (EC.1.6.99.2), also called DT:Diaphorase, is a flavoprotein that catalyses the two electron reduction of quinones and quinoinimines using NAD(P)H as electron donors. QR was shown to be an important chemoprotector agent against the carcinogenic effect of quinones. The 3-D structure of mouse Quinone Reductase (QR) in the presence of FAD was determined in two crystal forms by X-ray diffraction methods. Although QR activity is different for different species, the activities of human and mouse enzymes are very similar vis- a-vis the rat enzyme. Comparison of the mouse structure with the available rat model (Li et al. 1995) can provide a rationale for the observed differences. One aminoacid substitution in the FAD binding pocket has a marked effect in the positioning of FAD.

Li, R., Bianchet, M.A., Talalay, P. and Amzel, L.M. (1995) Proc. Natl. Acad. Sci. USA 92. 8846 - 8850

PS04.01.90 STRUCTURE DETERMINATION OF CHOLESTEROL OXIDASE CONTAINING COVALENTLY BOUND FAD. Kimberley Q. Yue, Nathalie Croteau and Alice Vrielink, Biochemistry Department & Montreal Joint Centre for Structural Biology, McGill University, Montreal, Quebec, Canada.

Cholesterol oxidase ia a bifunctional flavoenzyme which catalyses the oxidation of steroids containing a ß-hydroxyl group and the isomerization of the double bond at Δ^5-Δ^6 of the steroid ring. The protein is used clinically in the determination of serum cholesterol and for the assessment of arteriosclerosis. The structure of a form of the FAD prosthetic group non covalently bound to the enzyme has been solved and refined both in the presence and absence of a bound steroid substrate (1,2). A second form of the enzyme has been obtained from *Brevibacterium sterolicum* containing FAD covalently linked to His121 via the C8a group of the flavin isoalloxazine ring. Structural analyses of both forms of the enzyme will provide a unique opportunity to study the relationships between the flavin environment and their redox potential.

Single rod shaped crystals have been obtained for both the native enzyme containing covalently bound FAD and the His121Ala mutant. These crystals are grown by vapour diffusion