Old yellow enzyme (OYE) (EC 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 2.0 Å 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 P212121 (a=70Å, b=96Å, c= 151Å) and diffract to 2.5 Å 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.


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 benzoyl breaks 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 carbonation 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. Wilson1, Marta Ferraroni2, Pier Luigi Orioli3, Wojciech R. Rymiewski1 & Stefano Mangani1, European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany; 2Department of Chemistry, University of Siena, Pian dei Mantellini 44, I-53100 Siena, Italy; 3Department of Chemistry, University of Florence, via G. Capponi 7, I-50121 Firenze, 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 Vso = 4.5 Å3/dal, from which the solvent content can be estimated at 75%. Crystal structures have been solved of the unliganded enzyme and in complex with inhibitors, azide and thiocyanate. 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 released by the solvent. In addition, the crystal structure of reduced SOD and its complexes with copper 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 outer sphere 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 hydrogen peroxide. Different results are 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. Bianchetti1, Margarita Faigl1, Shiuan Chen2, Paul Talalay3 and L. Mario Amzel1, Dept. of Biophysics & Physical Chemistry, Johns Hopkins School of Medicine, Baltimore, MD, 21205, Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010; 3Dept. 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 quinonimines 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-à-vis the rat enzyme. Comparison of the mouse structure with the available rat model (L i 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.

**PS04.01.90 STRUCTURE DETERMINATION OF CHOLESTEROL OXIDASE CONTAINING COVALENTLY BOUND FAD.** Kimberly Q. Yue1, Nathalie Croteau and Alice Vriens, Biochemistry Department & Montreal Joint Centre for Structural Biology, McGill University, Montreal, Quebec, Canada.

Cholesterol oxidase is a bifunctional flavoenzyme which catalyses the oxidation of steroids containing a δ-hydroxyl group and the isomerization of the double bond at Δ-5-6 of the steroid ring. The protein is used clinically in the determination of serum cholesterol and for the assessment of atherosclerosis. 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. Some 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.