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

using the hanging drop technique. The precipitant conditions are 12% PEG8K, 75mM $MnSO_4$, 100mM cacodylate pH 5.2. In order to obtain large single crystals, a temperature difference between the nucleation event and the growth event is essential. The space group of cholesterol oxidase is monoclinic $P2_1$. The cell dimensions are a=77.6Å, b=125.7Å, c=81.5Å and ß=109.1° with two molecules per asymmetric unit. X-ray data collection is carried out by flash-cooling the crystals to 115K in a nitrogen stream. One heavy atom derivative has been obtained by soaking the crystals in 0.5mM $K_2Pt(CN)_4$. Further screening for heavy atom derivatives is in progress.

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PS04.01.91 THE STRUCTURE OF N-ACETYLNEURAMINATE LYASE IN COMPLEX WITH INHIBITORS: A NEW SUB-FAMILY OF ALDOLASES. M.C. Lawrence[1], J.A.R.G. Barbosa[1], B.J. Smith[1], N.E. Hall[1], P.A. Pilling[1], H.C. Ooi[2] & S.M. Marcuccio[2]. [1]Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia and [2]Biomolecular Research Institute, Private Bag 10, Clayton South MDC, Clayton South, Victora 3169, Australia

We present here the structure of N-acetylneuraminate lyase in complex with a known inhibitor hydroxypyruvate [1] and in complex with pyruvate via borohydride reduction. In both instances pyruvate is revealed covalently bound to the active site residue Lys-165. As the aldol cleavage reaction characteristic of this enzyme involves intermediate Schiff base formation between enzyme and substrate, the bound conformation of pyruvate presumably mimics that of the reaction intermediate state. The structures reveal in particular the interaction between the carboxylate group and the enzyme, the stereochemistry of the Schiff base complex and the role of the conserved residue Tyr-137 in substrate binding.

The structure of N-acetylneuraminate lyase [2] is similar to that of dihydrodipicolinate synthase [3] and the respective *E. coli* enzymes share a 24% sequence identity. Both sequences are similar to a third protein, MosA, of uncertain function and structure. We now show that two further enzymes (5-keto-4-deoxyglucarate dehydratase and trans-O-hydroxy-benzylidene-pyruvate hydratase-aldolase) are also members of this family. These enzymes all share pyruvate as either a product or substrate and we conclude that they share an active site structure and reaction pathway similar to that determined above for N-acetylneuraminate lyase. We predict these latter enzymes also to be $(\alpha/\beta)_8$ barrels and propose that they form a closely related aldolase sub-family.

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3. Mirwaldt, C. et al (1995) J. Mol. Biol. **246**, 227-239

PS04.01.92 CRYSTAL STRUCTURE OF PECTIN LYASE B DETERMINED BY MOLECULAR REPLACEMENT. Jacqueline Vitali[1], Brian M. Schick[1], Harry Kester[2], Jaap Visser[2], and Frances A. Jurnak[1], [1]University of California at Riverside, Department of Biochemistry, Riverside, CA 92521 and [2]Wageningen Agricultural University, Department of Genetics, NL-6703 HA Wageningen, The Netherlands

Aspergillus Niger produces four pectin lyases which are involved in the degradation of pectic substances. Pectin lyase B (pelB) is a member of this family. It contains 361 amino acids and is homologous to pectate lyases C and E whose structure has been determined. The overall sequence identity is 18.6% with pelE and 15.8% with pelC. When only the central parallel β– helix motif is considered, the sequence identity is 29.3% with pelE and 24.7% with pelC.

Crystals of pelB are orthorhombic, $P2_12_12$, with cell constants a = 83.70, b=88.80, c= 42.28 Å. Data were measured to 1.7 Å resolution, 33106 unique reflections, Rsym = 0.054. The structure was determined by the generalized molecular replacement method using XPLOR, 7.0 - 3.0 A data with F > 4*sigma, and a 5.0 - 24.0 A shell of integration in the rotation function. The search model consisted of most of the central β-helix motif of pelB (207 amino acids) in which all amino acids were replaced with Ala except for those common in the two proteins (67 amino acids). The solution corresponds to the top peak in both PC refinements and translational searches with signal to noise ratios of 1.33 and 1.22, respectively, the signal to noise ratio being defined as the ratio of the top peak to the top error peak, and has no steric conflicts with symmetry related molecules. After rigid body, Powell, and B refinement, the R and Rfree indices were 0.482 and 0.512, respectively. The electron density map has density for several of the missing side chains and for several of the missing loop regions in the expected positions, indicating the correctness of the solution. We are in the process of diffusing heavy atoms into the crystals in order to improve the phasing. Our progress on the refinement will be reported in the presentation.

PS04.01.93 ELUCIDATING THE MECHANISM OF TYROSINE PHENOL-LYASE Alfred Antson[1], Guy Dodson[1], Keith Wilson[1,2] and Tatyana Demidkina[3], [1]Department of Chemistry, University of York, York Y01 5DD, UK., [2]European Molecular Biology Laboratory (EMBL), c/o DESY, Notkestrasse 85, D-2000 Hamburg 52, Germany, [3]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov St., Moscow 117984, Russia

To understand chemical and structural events associated with the catalysis by tyrosine phenol-lyase (TPL) we performed X-ray studies of several complexes of this enzyme with cofactor (pyridoxal 5'-phosphate) and substrate analogues. TPL catalyses reversible ß-elimination of L-tyrosine to produce pyruvate, phenol and ammonia. This reaction goes through a number of intermediate steps which involve two enzyme-associated bases that abstract protons from the substrate Cα and phenol hydroxyl. TPL requires K+ for activity and in addition to physiological reaction catalyses ß-elimination of a number of ß-substituted amino acids and also the racemisation of alanine. In our study we used the X-ray structure of the apo enzyme as a crystallographic matrix for structure solution of different TPL complexes. The monovalent cation binding site has been derived from difference Fourier maps between X-ray data from apo enzyme crystals soaked with Cs+ and K+. The structure! of the holo enzyme, obtained by co-crystallisation with pyridoxal 5'-phosphate, has been refined to a crystallographic R-factor of 17.7 % (Rfree=20.5 %) at 1.9 Å resolution. X-ray data have been collected from freeze-trapped complexes of TPL with L-Alanine and with L-Alanine + 4-hydroxypyridine. The last two complexes were prepared by soaking the holo enzyme crystals and are thought to represent the quinonoid intermediate that forms after the abstraction of the Ca proton. Refinement of these structures is presently underway. Details of the ligand interactions and the possible catalytic mechanism will be presented.