PS04.01.76 STRUCTURE OF THE NATIVE CYSTEINE-SULFENIC ACID REDOX CENTER OF STREPTOCOCCAL NADH PEROXIDASE. Joanne I. Yeh§, Al Claiborne‡, and Wim G.J. Hol§, § Biomolecular Structure Center, Department of Biological Structure and Howard Hughes Medical Institute Box 357742, University of Washington, Seattle, Washington 98195-7742. ‡ Department of Biochemistry, Wake Forest University Medical Center, Winston-Salem, North Carolina 27157.

The flavoprotein NADH peroxidase (NPX) from *Streptococcus faecalis* represents one of two known flavin-dependent hydroperoxidases. Its flavin-linked function is chemically similar to flavoprotein disulfide reductases such as glutathione reductase. However, in NPX, an unusual, stabilized cysteine-sulfenic acid (Cys-SOH) is the redox center, in contrast to the redox active disulfide group of glutathione reductase. Indirect evidence for the existence of the Cys-SOH moeity resulted from metabolic labeling studies and FAB-mass spectrometric analysis of an active-site cysteinyl peptide (Poole and Claiborne, 1989). However, the initial x-ray crystal structure of the wild-type peroxidase refined at 2.16 Å resolution revealed that the Cys42 had been oxidized to the inactive sulfonic-acid form (Cys-SO_3H) (Stehle *et. al*, 1991).

In order establish the existence of the Cys-SOH form of the NPX, the x-ray crystal structure of the peroxidase with its native Cys42-sulfenic acid redox center was determined. To obtain the native, unoxidized enzyme, a strategy combining reduced exposure to ambient oxygen and data collection at -160°C was used. The structure containing the native, redox active site conclusively establish the existence of the Cys-42 sulfenic acid moiety as the functional nonflavin redox center of the peroxidase and provide the first structure for any naturally-occurring protein-sulfenic acid.

Poole, L.B. and Claiborn, A. (1989) *J. Biol. Chem.* **264**. 12322-12329.
Stehle, T., Ahmed, S.A., Claiborne, A., and Schultz, G.E. (1991). *J. Mol. Biol.* **221**, 1325- 1344.

PS04.01.77 CRYSTAL STRUCTURE OF RAT LIVER NADPH-CYTOCHROME P-450 REDUCTASE. Ming Wang, David L. Roberts, Rosemary Paschke, Thomas Shea§, Bettie Sue S. Masters§ and Jung-Ja P. Kim , Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226 and §Department of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

NADPH-cytochrome P-450 reductase (CPR) catalyzes the transfer of electrons from NADPH to all known microsomal cytochromes P450. CPR consists of a large 70 kDa cytoplasmic domain that contains both FMN and FAD as well as a NADPH binding domain, and a small, ~50 residue hydrophobic N-terminal domain responsible for anchoring the protein to the endoplasmic reticulum or nuclear envelope of most eukaryotic cells. Electron transfer proceeds from NADPH to FAD to FMN and finally to the heme of cytochrome P-450.

We have crystallized the cloned cytoplasmic domain of rat CPR and have collected a native data set to 2.6 on a Rigaku R-AXIS image plate detector with R_{sym} = 7.1%. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters a= 103.28, b= 116.18 and c= 119.77 Å. The V_m is 2.6Å^3/dalton, with two molecules per asymmetric unit. The two molecules are related by a local two fold axis which is almost parallel to c.

The structure has been solved to 3.0 by MIR, with four heavy atom derivatives and a figure of merit was 0.595. The phases were extended and improved by using solvent flattening and local-two-fold averaging to 3 Å. The model was built by using the TURBO package and refined by XPLOR. The current R factor is 27.8%. Further refinement is in progress.

This work was supported by NIH grant GM29076 (JJPK).

PS04.01.78 CRYSTAL STRUCTURE OF COPPER AMINE OXIDASE OF YEAST. Rongbao Li[1], Longying Chen[1], Goeffery Boyd[1], Daiying Cai[2], Judith Klinman[2] and F. Scott Mathews[1]. [1]Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110; [2]Department of Chemistry, University of California, Berkeley, CA 94720.

The copper-containing amine oxidase from the yeast *Hansenula polymorpha* has been solved by molecular replacement. It is a ubiquitous dimeric enzyme that catalyze the oxidative deamination of primary amines by molecular oxygen to the corresponding aldehydes, ammonium and hydrogen peroxide. Copper amine oxidases are a novel group of quinoenzymes that contain a covalently bound redox cofactor, 2,4,6-trihydroxyphenyl-alanine quinone (topa quinone, or TPQ) generated by post-translational modification of a single tyrosine side-chain. The enzymes allow the microorganism to use the appropriate amines as a carbon and nitrogen source for growth. In animals the enzymes modulate the levels of amines, thereby being implicated in cell growth, development and detoxication. Purified enzyme, expressed in *Saccharomyces cerevisiae* (Danying Cai and Judith P. Klinman, *Biochemistry*, 1994, **33**, 7647-7653) was crystallized using vapor diffusion. The crystals are orthorhombic, with space group symmetry $P2_12_12_1$ and unit cell dimensions of a = 137.9 Å, b = 145.8 Å, c = 234.1 Å. Using a cryogenic cooling system the native protein crystals diffract x-rays to 2.3 Å resolution; however, due to the long unit cell axis the data were only collect to 2.9 Å with 97% completeness. There are two dimers in the asymmetric unit. The self-rotation function indicated that each dimer has two-fold symmetry and the two dimers are related by a two-fold axis. The molecular replacement solution is in good agreement with the self-rotation function results. The enzyme is about 28% homologous to copper amine oxidase of *E. coli* ; molecular replacement using the structure of this protein as a search probe (M. R. Parsons, M. A. Convery, C. M. Wilmont, K. D. S. Yadav, V. Blakeley, A. S. Corner, S. E. V. Philips, M. J. McPherson and P. F. Knowles, *Structure* 1995, **3**, 1171-1179) has yielded a starting model which contains 90% of the residues. Refinement of this model is proceeding in combination with SIR determined phases.

PS04.01.79 THE CRYSTAL STRUCTURE OF A TYPE II DEHYDROQUINASE FROM *MYCOBACTERIUM TUBERCULOSIS*. D.G.Gourley,[1] J.R.Coggins[1], A.R. Hawkins[2], and N.W. Isaacs[1]. [1]Departments of Biochemistry and Chemistry University of Glasgow, Glasgow G128QQ,UK; [2]Department of Biochemistry and Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH,UK.

Dehydroquinases (DHQases) are enzymes that catalyse the reversible dehydration of 3-dehydroquinate to 3-dehydroshikimate. The DHQase reaction is a common step in two important pathways: the shikimate pathway for aromatic biosynthesis and the catabolic pathway for quinate metabolism. Some of the aromatic compounds made by the shikimate pathway are essential to these organisms and thus contains viable targets for the design of anti-microbial drugs and herbicides. Two classes of DHQase exist with different biochemical and biophysical properties. The Type I DHQase is found in the shikimate pathways of plants, fungi and most bacteria but has never been found in the catabolic pathway. Type II DHQase has been found in the catabolic pathway and in the shikimate pathway of four organisms including *Mycobacterium tuberculosis*.

Crystals of type II DHQase from *Mycobacterium tuberculosis* belong to spacegroup F23 with a=b=c=127.8 and diffract to 1.9 Å resolution. The structure has been determined to 2.9 Å with MIR phases calculated from two derivatives followed by density modification. The fold has been identified as α/β type and is similar to the fold found in P-loop containing nucleotide triphosphate hydrolases such as $p21^{ras}$ (Pai *et al.*, Nature **341**, 209-214).