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

The flavoprotein NADH peroxidase (NXP) 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 NXP, 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 moiety 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 sulfenic-acid form (Cys-SO\(_2\)H) (Stehle et al., 1991).

In order establish the existence of the Cys-SOH form of the NXP, 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 establishes the existence of the Cys-42 sulfenic acid moiety as the functional nonflavin redox center of the peroxidase and provides the first structure for any naturally-occurring protein-sulfenic acid.


PS04.01.77 CRYSTAL STRUCTURE OF RAT LIVER NADPH-CYTOCHROME P-450 REDUCTASE. Ming Wang, David L. Roberts, Rosemary Paschke, Thomas Shea, Betty Sue S. Masters, and Jung-Fa 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 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 Å on a Rigaku R-axis rotating anode generator. We have refined the native protein crystals diffract x-rays to 2.3 Å resolution. The current R factor is 27.8%. Further refinement is in progress.

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PS04.01.78 CRYSTAL STRUCTURE OF COPPER AMINE OXIDASE OF YEAST. Rongchao Lii, Longying Chen, Geoffrey Boyd, Daiying Cai, Judith Kliman, F. Scott Mathewa, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110; 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 catalyzes 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 quinonenzymes that contain a cofactor tetrahydropyridoxal-phosphate (TPD or TPO) that has been 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 detoxification. Purified enzyme, expressed in Saccharomyces cerevisiae (Daiying Cai and Judith P. Kliman, Biochemistry, 1994, 33, 7647-7653) was crystallized using vapor diffusion. The crystals are orthorhombic, with space group P2\(_1\)2\(_1\)2\(_1\) 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 to 2.9 Å with 97% completeness. There are two dimers in the asymmetric unit. The self-rotation function results indicate 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. Phillips, 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, J.R. Coggins, A.R. Hawkins, and N. Wang. 1Department of Biochemistry and Chemical Genetics, University of Glasgow, Glasgow G128QQ, UK; 2Department of Biochemistry and Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK.

Dehydroyquinases (DHQases) are enzymes that catalyse the reversible dehydration of 3-dehydroquinate to 3-dehydroshildmate. 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 space group P2\(_3\) with a = b = c = 127.8 Å and 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 a \(\alpha/\beta\) type and is similar to the fold found in P-loop containing nucleotide triphosphate hydrolases such as p21Ras (Pai et al., Nature 341, 209-214).