03-Crystallography of Biological Macromolecules

MS-03.04.06 WHAT DO WE KNOW ABOUT CYTOCHROME C PEROXIDASE? J. Kraut, Department of Chemistry, University of California/San Diego, La Jolla, California.

The purpose of this talk is to give a brief overview of what is known to date about cytochrome c peroxidase (CCP). I hope it will serve as a background briefing for H. Pelletier's report in MS 03.5 on the structures of two CCP:cytochrome c complexes.

CCP is a heme-containing enzyme which occurs between the inner and outer membrane of yeast mitochondria. It catalyzes the overall net reaction

$$2Cc(II) + H_2O_2 + 2H^+ \rightarrow 2Cc(III) + 2H_2O$$

that is, the oxidation of two molecules of reduced cytochrome c by a molecule of hydroperoxide. Because CCP is small (294 residues) and, unlike other peroxidases has an electron transfer protein as its substrate, it may be a simple model for the large multisubunit membrane-bound enzymes of respiration and photosynthesis.

The heme environment of CCP crudely resembles that of myoglobin in that both a distal and a proximal histidine are present, but the similarity stops there. Key features of the structure that are implicated in the peroxidase mechanism include the distal-side Arg-48 which is involved in heterolysis of the peroxide molecule along with the distal His-52; and the proximal-side Trp-191 with it's indole ring parallel to the proximal imidazole and hydrogen bonded to the buried Asp-235. The latter sub-assembly is important for electron transfer to cytochrome c. In its resting state the CCP heme iron is Fe(III), and the two-equivalent oxidized intermediate contains both an oxyferryl heme center, Fe(IV)=O, and an EPR-active radical at Trp-191. Several mutants have been designed to test these ideas and their structures and kinetic properties have been determined.

MS-03.04.07 ENGINEERING METAL SPECIFICITY IN GLUCOSE (XYLOSE) ISOMERASE. By Joël Janin, Laboratoire de Biologie Structurale, CNRS and Université Paris-Sud, 91405-Orsay, France

Xylose isomerase (XI) converts xylose to xylulose and glucose to fructose. It is used by the food industry to prepare isoglucose, a mixture that is sweeter than pure glucose, and usually called glucose isomerase. Its activity requires divalent cations (Mg^{++}, Co^{++} or Mn^{++}). Protein engineering was carried out on XI from the bacterium *Actinoplanes missouriensis* in a collective effort led by Pr. S. Wodak (Free University, Brussels) and Plant Genetic Systems (Gent and Brussels, Belgium). Results lead among other things to a better understanding of the catalytic role of metals. The X-ray structure of a trigonal crystal form containing the whole 172K tetramer in its asymetric unit was solved in Orsay using synchrotron radiation from LURE-DCI. The model was refined to a R factor of 0.15 at 2.2 Å resolution[1], and further X-ray studies were done on a series of complexes of wild type and mutant enzymes with substrates, linear and cyclic inhibitors, and a variety of cations. Twenty structures with comparable resolution and refinement statistics are now available for *Actinoplanes missouriensis* XI[2].

Each XI subunit carries two cation sites 5 Å apart. Sugar substrates bind to both cations through their keto and hydroxyle oxygens. Metal binding maintains the sugar in an open conformation and plays an essential part in isomerization. While significant activity is retained when potential base catalysts are removed by site directed mutagenesis, mutating metal ligand residues at either site deeply perturbs catalysis[3]. This supports a mechanism based on 1-2 hydride transfer driven by the electric field of the metal cations and the polarization of the C-O bonds. The substitution of residues close to, but not at, the active site leads to interesting effects. The E186Q mutation removes a charge near a cation site[4]. The mutant enzyme has low activity with Mg^{++} or Co^{++}. It is fully active with Mn^{++}, and its pH optimum is shifted down by about 1.5 pH units. The X-ray structure of the mutant protein was done in the presence of xylose and either Mg^{++} or Mn^{++}. The E186Q-Mn^{++} structure is essentially identical to the wild type, but in the E186Q-Mg^{++} complex, major changes at the active site affects the second metal binding site, but not substrate binding. These changes are clearly related to the presence of the engineered amide group of Q186. Amidation may simulate protonation of E186 in the wild type enzyme, leading at low pH to the same conformation change as seen in the E186Q-Mg^{++} structure.

1 N.T. Mrabet, A. van den Broek, I. van den Brande, P. Stanssens, Y. Laroche, A.M. Lambeir, G. Matthyssens, J. Jenkins, M. Chiadmi, H. van Tilbeurgh, F. Rey, J. Janin, W.J. Quax, I. Lasters, M. de Maeyer & S. Wodak (1992) Biochemistry 31, 2239-2253

2 J. Jenkins, J. Janin, F. Rey, M. Chiadmi, H. van Tilbeurgh, I. Lasters, M. de Maeyer, D. van Belle, S.J. Wodak, M. Lauwereys, P. Stanssens, N.T. Mrabet, J. Snauwaert, G. Matthyssens & A.M. Lambeir (1992). Biochemistry 31, 5449-5458

3 A.M. Lambeir, M. Lauwereys, P. Stanssens, N.T. Mrabet, J. Snauwaert, H. van Tilbeurgh, G. Matthyssens, I. Lasters, M. de Maeyer, S.J. Wodak, J. Jenkins, M. Chiadmi & J.Janin (1992). Biochemistry 31,5459-5466

4 H. van Tilbeurgh, J. Jenkins, M. Chiadmi, J. Janin, S.J. Wodak, N.T. Mrabet & A.M. Lambeir (1992). Biochemistry 31, 5467-5471

PS-03.04.08 **2.8Å CRYSTAL STRUCTURE OF CATALASE HPII FROM E.COLI.** By J.Bravo*, J.Tormo,N.Verdaguer,I.Fita Dept. Eng. Química ETSEIB-UPC Diagonal 647 08028 Barcelona Spain. C.Betzel EMBL-Outstation Hamburg, Germany and J.Switala and P.C.Loewen, Univ. Manitoba, Winnipeg,Canada.

Green plate like crystals, reaching sizes of about 0.8x0.5x0.1mm and suited for high resolution studies, have been obtained for catalase HPII from E.Coli. Crystals with the same morfology have also been produced for mutants N201H (brown crystals) and N201A. These crystals appeared in a few days with the hanging-drop vapor diffusion method at room temperature and using PEG 3350 and LiCl as precipitans at pH 9.0.

Crystals from native HPII difract at least to 2.2Å and are quite stable to X-ray radiation. A complete data set (98% of the unique reflections at 2.8Å) has been collected using an Image Plate (MARESEARCH) area detector. In spite of the molecular weight differences (84 KDa and 57KDa per subunit for HPII and BLC respectively), the initial analysis of the electron density maps shows that the molecular structure has a high similarity with the tetrameric beef liver catalase (BLC).The structure determination will be presented.

