When supplied with suitable electron donors, SiRHP catalyzes the six-electron reduction of sulfite to sulfide, and nitrite to ammonia, without releasing any detectable intermediates. The protein's internal twofold symmetry relates geneduplicated modules that have diverged to bind different cofactors for assembly of the active center. SiRHP's unique active center consists of a siroheme, (an unusual irontetrahydroporphyrin of the isobacteriochlorin family), coupled structurally and electronically to an [4Fe-4S] cluster via a cysteinate sulfur bridge.

The ligand binding site, on the siroheme's distal face, has been optimized for electrophilic catalysis, and has been constructed to provide preferred locations for recognizing the functional groups of ligands with varied size, shape and charge.

Complex interplays relate the electronic states of the prosthetic groups, the interactions of ligands with the cofactors and the protein, and the structural coupling of the cofactors to the protein moiety. Correlated changes occuring in the electronic state of the siroheme iron and the conformations of active-site residues and exposed loops sequester and activate the substate sulfite for reduction.

MS04.02.03 CRYSTAL STRUCTURE OF A PCB—DEGRADING DIOXYGENASE, THE BphC ENZYME FROM A PSEUDOMONAS SP. Y. Mitsui, T. Senda, M.Fukuda, Department of BioEngineering, Nagaoka University of Technology, Nagaoka, Niigata 94-21, Japan

Polychlorinated biphenyls (PCBs) are widely distributed environmental pollutants. Because of their chemical stability, they are hard to decompose. Pseudomonas sp. strain KKS102 is one of the PCB-degrading microorganisms and the "BphC" enzyme (2,3-dihydroxybiphenyl dioxygenase) is a key enzyme in its PCB degrading pathway. The BphC enzyme has been characterized as one of the extradiol type dioxygenases which, when active, have one ferrous iron in their active sites. It is an oligomeric enzyme made up of eight identical subunits each of 292 amino acid residues. The total molecular weight is ca. 250kDa.

The structure was solved by MIR method at 1.8 Å resolution(1). Crystal structures of the complexes with its substrates, 2,3-dihydroxybiphenyl (2,3-DHBP) or 3-methylcatechol (3-MCT), have also been solved(2). In these crystals, the Fe ion was found to be in a ferric state explaining the existence of the intact substrates in the active sites.

The Fe ion is coordinated by the side chain atoms of His145, His209, Glu260 and two solvent molecules forming a square-pyramid. In the complexes with its substrates, two hydroxyl groups from the catechol ring moiety of the substrates (rather than the solvent molecules) were bound to the Fe ion forming a trigonal bipyramid. The van der Waals surface of the active site and that of the bound substrate are almost complimentary to each other. Thus the basic coordination geometry of the BphC enzyme in complex with its substrate must be mainly determined by the stereochemical effect and may be retained even if the Fe oxidation state were changed. Apparently in accord with this notion, the polyhedra formed by the Fe ion (in a ferrous state) and the three equivalent protein ligands found in the substrate—free crystal structure of a similar enzyme (having ca.66 % amino acid sequence homology), which was solved later under unaerobic condition(3), was superimposable on the present polyhedra (involving a ferric Fe ion) with a rms deviation of only 0.19 A(2). Details of the present structure and further studies on several mutated enzymes will be given in a poster presented by T.Senda et al.

(1) K. Sugiyama, T. Senda et al. Proc. Japan Acad. 71B, 32 -35 (1995).
(2) T. Senda, M. Fukoda et al. J.Mol. Biol. 255, 735 - 752 (1996).
(3) S. Han, L. D. Eltis, K. N. Timmis et al. Science 270, 976 - 980 (1995).

MS04.02.04 OXIDIZED AND REDUCED NITRITE REDUCTASE WITH AND WITHOUT NITRITE BOUND Elinor T. Adman, Michael E.P.Murphy, Stewart Turley, Mutsuko Kukimoto, and Makoto Nishiyama. Dept of Biological Structure, University of Washington, Seattle WA, 98195-7420 and Dept of Biotechnology, University of Tokyo, Yayoi 1-1-1 Bunkyo-ku, Tokyo 113 JAPAN.

Dissimilatory nitrite reductase is a trimeric copper-containing protein in *Alcaligenes faecalis* and *Achromobacter cycloclastes*. NO is the immediate product and is an intermediate in the dissimilatory denitrification pathway, in which nitrate is completely reduced to N_2. Data from crystals of *Alcaligenes faecalis* NIR at -160° C in its oxidized and reduced forms, and with nitrite soaked into crystals, show that the reduced form binds ligands much less tightly than the oxidized form. Previouly published results have shown that the electron transfer partner of nitrite reductase, pseudoazurin, donates electrons to NIR via the Type I Cu site. Electrons are then transferred internally to the Type II Cu site, normally liganded by three histidines and a solvent. A more weakly bound ligand in the reduced form suggests that nitrite first replaces the solvent ligand in the oxidized form, modifying the redox potential of the active site Type II copper so that electrons are then transferred from the Type I site.

Four data sets were collected at -160° C on an R-axis II image plate using crystals cryoprotected in PEG, glycerol and methanol. Oxidized crystals were soaked in nitrite at room temperature and then cooled. Crystals reduced with ascorbate were cooled to -40° C before nitrite was added. The average cell dimensions were a=61.84 Å, b=102.6 Å, c=146.2 Å, space group $P2_12_12_1$, 5% smaller in volume than the room temperature cell. Difference maps using phases from a rigid body refinement of PDB coordinates AFN2 (R=0.29, 10-2 Å) revealed the clearest view of nitrite in the active site when amplitudes from oxidized + nitrite crystals minus amplitudes from reduced crystals were used.

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MS04.02.05 STRUCTURE OF HUMAN GLYOXALASE I, A ZINC ENZYME, SOLVED BY MIR METHODS. A.D. Cameron, B. Olin, M. Ridderström, B. Mannervik & T.A. Jones. Departments of Molecular Biology and Biochemistry, Uppsala University, BMC, Box 590, S-751 24, Uppsala, Sweden

The glyoxalase system catalyses the conversion of methyglyoxal into D-lactic acid using glutathione as coenzyme. It is found at all levels of evolution in the cytosol of cells and has been targetted for the development of anti-cancer drugs and antimalarial agents. There are two enzymes involved in the pathway, glyoxalase I and glyoxalase II. Glyoxalase I catalyses the conversion of the hemithioacetal formed from the non-enzymatic reaction between methylglyoxal and reduced glutathione, into S-D-lactoylglutathione. Glyoxalase II, in turn catalyses the hydrolysis of the product of the first reaction to form D-lactic acid and regenerate the reduced glutathione.

We have solved the structure of human glyoxalase I by MIR coupled with four-fold non-crystallographic averaging and are currently refining the structure against data from an inhibitor complex extending to a resolution of 2.2Å. The enzyme is a dimer of molecular weight 50,000 and contains one zinc ion per monomer which is essential for activity. The inhibitor, benzyl glutathione, is clearly defined in the electron density. It is situated in the middle of an eight stranded beta-sandwich reminiscent, of the retinol binding proteins, and juxtaposed to the zinc ion. There is no structural homology with other glutathione binding sites. As expected from EPR and EXAFS studies there are four protein ligands to the zinc (His, Glu, Glu and Gln), and one water molecule