

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 gene-duplicated 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 iron-tetrahydroporphyrin 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 occurring in the electronic state of the siroheme iron and the conformations of active-site residues and exposed loops sequester and activate the substrate 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 complementary 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 anaerobic condition(3), was superimposable on the present polyhedra (involving a ferric Fe ion) with a rms deviation of only 0.19 Å(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. Fukuda 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<sub>2</sub>. 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. Previously 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<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, 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.

This work has been supported by NIH grant GM31770 and the Medical Research Council of Canada.

**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 methylglyoxal into D-lactic acid using glutathione as coenzyme. It is found at all levels of evolution in the cytosol of cells and has been targeted for the development of anti-cancer drugs and anti-malarial 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

which has been implicated in catalysis. What was completely unsuspected, however, is that two of the ligands to the zinc come from one monomer and two from the other. Indeed, the whole of the active site is formed only on dimerisation. Each monomer contains two structurally very similar domains. Of the eight strands making up the beta-sandwich, four strands are from the N-terminal domain of one monomer and four from the C-terminal domain of the other. What is most remarkable, however, is that if the N- and C-terminal domains are superimposed, the two zinc ligands of one domain overlap almost exactly with the those of the other domain. This has important implications for the evolution of the enzyme.

**MS04.02.06 CRYSTAL STRUCTURE OF THE SELENOCYSTEINE, MOLYBDOPTERIN AND 4Fe4S CLUSTER CONTAINING ENZYME FORMATE DEHYDROGENASE-H.** Jeffrey C. Boyington<sup>1</sup>, Vadim Gladyshev<sup>2</sup>, Thressa C. Stadtman<sup>2</sup> and Peter D. Sun<sup>1</sup>, <sup>1</sup>Laboratory of Molecular Structure, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, <sup>2</sup>Laboratory of Biochemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

Formate dehydrogenase-H is a key redox component of the formate hydrogen lyase complex of *E. coli*, oxidizing formate to carbon dioxide with the release of two protons and two electrons under anaerobic conditions. This 80 kDa protein contains a 4Fe4S cluster and a molybdenum atom that is coordinated by two molybdoterin guanine dinucleotide (MGD) cofactors and an essential selenocysteine residue. The three-dimensional structure of both the reduced (Mo<sub>IV</sub>, 4Fe4S<sub>red</sub>) and oxidized (Mo<sub>VI</sub>, 4Fe4S<sub>ox</sub>) forms of formate dehydrogenase-H have been determined by x-ray crystallography using multiple isomorphous replacement and multiwavelength anomalous scattering methods to a resolution of 2.3 Å and an R=21.9% for the reduced form and a resolution of 2.8 Å and an R=20.0% for the oxidized form. Both structures are comprised of four  $\Sigma/\Sigma$  domains including two MGD cofactor binding domains that are related by a pseudo two-fold rotation axis. Examination of the structure of the oxidized form of the enzyme reveals a 27 degree rotation of one of the MGD cofactors bound to the molybdenum relative to the reduced form with comparatively little movement observed in the rest of the protein. This rotation creates a strained molybdenum coordination geometry in the catalytically active oxidized form so that it is poised to accept the substrate formate as an additional molybdenum ligand. This is the first three-dimensional protein structure of a selenium and molybdenum containing enzyme determined to date. A catalytic mechanism is proposed based on both oxidation states of the enzyme, which incorporates all three of the cofactors revealing a probable electron flow.

**MS04.02.07 CRYSTAL STRUCTURE OF DIMETHYLSULFOXIDE REDUCTASE DEFINES A NEW FAMILY OF OXOTRANSFERASES CONTAINING THE MOLYBDENUM COFACTOR.** H. Schindelin, C. Kisker, D. C. Rees, Division of Chemistry and Chemical Engineering, 147-75CH, California Institute of Technology, Pasadena, CA 91125, USA

Enzymes containing the molybdenum cofactor (Mo-co) constitute a class of oxotransferases which are widespread in organisms ranging from bacteria, to higher plants and animals, including humans. These enzymes catalyze the transfer of an oxogroup between the substrate and water in a two-electron redox reaction. Dimethylsulfoxide (DMSO) reductase is found in a variety of bacteria, including *Escherichia coli*, *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*. In these organisms, the enzyme serves as a terminal electron acceptor during anaerobic growth in the pres-

ence of DMSO. The crystal structure of DMSO reductase from *Rhodobacter sphaeroides* has been determined in the oxidized, Mo(VI), and reduced, Mo(IV), forms and contains a Mo-co comprising two molybdopterine guanosine dinucleotides as organic components. The fold of the polypeptide chain can be roughly divided into four domains, which, with the exception of the first domain, are all involved in cofactor binding. The second and third domains share structural homology, despite the absence of any sequence homology and each coordinates one guanosine. The fourth domain which mostly interacts with the two pterin moieties, has an unusual topology, since it consists of a six-stranded  $\beta$ -barrel containing parallel as well as anti-parallel  $\beta$ -strands. The two molybdopterine guanosine dinucleotides asymmetrically coordinate the molybdenum with their dithiolene sulfurs. Notably, a serine sidechain oxygen is coordinated to the metal in both oxidation states, while in the reduced enzyme, in addition to the absence of the oxo-ligand present in the oxidized form, one of the dithiolene sulfurs no longer remains coordinated to the molybdenum. Differences in the coordination of the molybdenum between the oxidized and the reduced states provide valuable clues about the reaction mechanism of this enzyme. Sequence comparison of DMSO reductase with enzymes containing a molybdopterine guanine dinucleotide suggests similarities in both the protein architecture and the presence of two molybdopterins, thus defining DMSO reductase as the archetype of this group of bacterial oxotransferases.

**PS04.02.08 STRUCTURE OF THE ALDEHYDE OXIDOREDUCTASE FROM DESULFOVIBRIO GIGAS: A MEMBER OF THE XANTHINE OXIDASE PROTEIN FAMILY.** Archer, M.<sup>1</sup>, Romco, M. J.<sup>1,2</sup>, Duarte, R.<sup>3</sup>, Moura, I.<sup>3</sup>, Moura, J.J.G.<sup>3</sup>, LeGall, J.<sup>4</sup>, Hof, P.<sup>5</sup>, Huber, R.<sup>5</sup>, ITQB, Apt 127, 2780 Oeiras, Portugal, <sup>2</sup>IST, 1000 Lisboa, Portugal, <sup>3</sup>FCT-UNL, 2785 Monte Caparica, Portugal, <sup>4</sup>University of Georgia, Athens, GA 30602, USA, <sup>5</sup>MPI, D-82152 Martinsried, Germany

The crystal structure of the Aldehyde Oxidoreductase (MOP) has been solved by the MIR method at 2.25 Å resolution and refined to an Rfactor of 16.5% [1]. MOP, isolated from the sulfate reducing bacterium *Desulfovibrio gigas* is a homodimer of 907 aminoacid residues per subunit. It contains a molybdopterine cofactor (mo-co) and two different [2Fe-2S] centers and is folded into four domains. The first two bind the two iron sulfur clusters and the others are associated with Mo-co. Mo-co is a molybdopterine cytosine dinucleotide and is deeply buried in the protein and accessible through a 15 Å deep tunnel. The Mo is pentacoordinated by two dithiolene sulfur atoms of the molybdopterine and three oxygen ligands. MOP oxidizes aldehydes to carboxylic acids. It belongs to an electron transfer chain, consisting of four proteins from *D.gigas* - flavodoxin, cytochrome c3 and hydrogenase - so that the oxidation of aldehydes may be linked to the generation of hydrogen. MOP sequence reveals homology to the xanthine oxidases. In xanthine oxidases (ca 1300 residues) the electron transfer occurs from the Mo site to a flavin group, which is absent in MOP. The crystal analysis of MOP shows the structure of Mo-co in detail and allows a first look at a member of the xanthine oxidase family. A structure-based catalytic mechanism for the xanthine oxidase family of molybdenum enzymes is proposed.

1. Romco, M. J., Archer, M., Moura, I., Moura, J. J. G., LeGall, J., Engh, R., Schneider, M., Hof, P., Huber, R., Science 270, 1170 (1995).