(residues ß9-ß22 and ß275-ß295) is missing in OASS. There are two additional surface helices in TRPSß (residues ß23-ß54) and an additional loop (ß260-ß266). The active site cleft of OASS is wider and therefore more exposed to the solvent. The hydrophobic channel for indole transport from the α to the ß active site is, not unexpectedly, missing in OASS. The dimer interface, however, is more or less conserved in the two enzymes.

The only cysteine residue of OASS (which is the residue following the active site lysine in the sequence) cannot be directly involved in the reaction mechanism, since it is completely buried and more than 10 Å away from the PLP cofactor. Further investigations on substrate binding and possible reaction mechanisms are planned.

1) Crystallization and Preliminary X-ray Data for the A-Isozyme of O-Acetylserine Sulfhydrylase from Salmonella typhimurium, Rao et al., JMB 231, 1130-1132 (1993)

PS04.01.118 CRYSTAL STRUCTURE OF AN ACTIVE FORM OF PORCIN TRYPSIN. A. Johnson, Vasantha Pattabhi and P.V.Sundaram+ Department of Biophysics, University of Madras, Guindy Campus, Madras-600025, INDIA, +Protein Engineering and Biomedical Research Voluntary Health Services Campus, Madras-6000113, INDIA

An active form of porcine trypsin has been crystallised from acetate buffer at pH 6.7 using 0.4M ammonium sulfate as a precipitant at 20° C. The crystals belong to P21 21 21 space group with cell dimensions a=47.07, b=53.82, c=77.7Å. Three dimensional data has been collected up to 1.8 Å resolution. The structure solution is by molecular replacement.Conformational comparisons of the active site residues with those of the inactive form of porcine trypsin will be presented.

PS04.01.119 CRYSTAL STRUCTURE OF CALCIUM-FREE C-TERMINAL DOMAIN OF SMALL SUBUNIT OF RAT CALPAIN. P. Grochulski*, H. Blanchard*, Y. Li*, J.S.C. Arthur+, J.S. Elce+, P.L. Davies+ & M. Cygler*. *Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec H4P 3R2. +Department of Biochemistry, Queen's University, Kingston, Ontario K7L 3N6

Calpain is a name given to a family of Ca^{2+}-dependent cysteine proteases. Although the physiological function of calpain is unknown, a role in signal transduction has been suggested. Rat calpain II is a heterodimer containing an 80 kDa catalytic subunit, and a 30 kDa regulatory subunit. The 21 kDa calcium-binding C-terminal domain of the small subunit has been expressed, purified and crystallized (Blanchard *et al.*). The crystal form depends on the presence or absence of calcium in the crystallization drop.

We report the crystal structure of the calcium-free form, space group $C222_1$, cell dimensions of a=67.6, b=73.1 and c=156.6 Å, with two molecules in the asymmetric unit. Due to nonisomorphism of heavy atom derivatives we have used the multi-wavelength anomalous dispersion (MAD) method to derive protein phases. Native crystals are very sensitive to mercurials, but a mercury derivative was obtained using a C60S mutant. Data for this mutant were collected using a rotating anode and synchrotron radiation at beamline X4A at the Brookhaven National Synchrotron Light Source (NSLS). These data were combined with data collected on the X12C beamline at NSLS for a crystal of selenomethionyl protein. Data collected at X4A for the mercury derivative indicated one major site and one minor site and allowed us to find the positions of all sixteen selenium atoms from cross Bijvoet difference Fourier and cross difference Fourier maps. The electron density maps calculated for the mercury crystal and selenomethionine crystal possessed similar features but neither were easy to interpret. Combination of phases from both sources resulted in a much better map. Almost a complete model was traced in a 2.5 Å combined MAD map. One monomer seems to be built from repeats of a basic unit consisting of two short and one long helix, suggesting a gene duplication.

Metalloenzymes

MS04.02.01 A UNIQUE ACTIVE SITE IN A ROBUST ENZYME. Evelyn Jabri, P. Andrew Karplus. Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY, 14853.

The structure of the nickel metalloenzyme urease from *Klebsiella aerogenes* was solved at 2.2Å resolution (1). The enzyme is a trimer of three (αβγ)-units, each consisting of four structural domains. The α-subunit contains the active site in an $(\alpha\beta)_8$-barrel domain which is homologous to the Zn-dependent enzymes adenosine deaminase and phosphotriesterase. The two active site nickels are 3.5Å apart and have nonstandard coordination geometry. Ni-1 has an unusual tricoordinate geometry whereas Ni-2 is pentacoordinate. Both ions are coordinated by a carbamylated lysine, $Lys^{\alpha 217}$, explaining why CO_2 is required for the activation of the apoenzyme. We have analyzed the 2.3Å resolution structure (R=19%) of the apoenzyme, and the 2.5Å resolution structures (R=17.9% and 18%, respectively) of the two catalytically impaired active site mutants, H219A and H320A. The final apoenzyme model lacks the CO_2 modification of the lysine and the two nickel ions. Otherwise, the structure of the apoenzyme is nearly identical to that of the holoenzyme, suggesting a high degree of preorganization which helps explain the tight binding of the nickel ions. The major change in the structure of H219A involves a conformational shift and ordering of the active site loop, and a small shift in the side chain of $Asp^{\alpha 221}$. This latter movement may contribute to the lower activity of H219A. In the structure of H320A, the catalytic water, primarily a Ni-2 ligand in the holoenzyme, shifts into a bridging position. This result shows that the nickel ligation is rather sensitive to the environment at the active site and provides an alternate explanation for the 10^5-fold lower activity of H320A. These results also show that urease is robust to the loss of nickel ions and active site mutations. Analysis of the tertiary/quaternary structure suggests that the stability of urease may be due to the burial of an unusually large fraction of its residues.

(1) Jabri, Carr, Hausinger, Karplus (1995) Science 268:998-1004.

MS04.02.02 CRYSTALLOGRAPHIC STUDIES OF THE MULTI-ELECTRON REDUCTIONS CATALYZED BY THE SIROHEME AND IRON-SULFUR CLUSTER CONTAINING ENZYME SULFITE REDUCTASE. Brian R. Crane, Lewis M. Siegel and Elizabeth D. Getzoff, Department of Molecular Biology, The Scripps Research Instituite, La Jolla California, 92037, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Reductions of the inorganic substrates sulfite and nitrite are important for the assimilation of sulfur and nitrogen into the biosphere and for the dissimilation of oxidized forms of these elements during anaerobic energy procurement.

To further understand the enzymatic redox chemistry involved in these processes we have characterized high-resolution crystallographic structures of the 64 kD E.coli NADPH sulfite reductase hemoprotein (SiRHP) in different oxidation states, and in complex with inhibitors, substrates, reaction intermediates and products.

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 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