(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 $(\alpha\beta\gamma)$-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 Institiute, 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.