

The crystallographic R-factor of current model is 19.2% for 69,264 unique reflections with $F_o > 2\sigma F$ in the range of 8.0 - 2.3 Å. The root mean square deviations from ideal stereochemistry are 0.008 Å for bond lengths and 1.095° for bond angles. The structural basis for the extreme thermostability of this enzyme will be discussed.

PS04.01.114 CRYSTALLOGRAPHIC STUDIES ON THE BIFUNCTIONAL PTERIN-4A-CARBINOLAMINE DEHYDRATASES FROM HUMAN LIVER AND PSEUDOMONAS AERUGINOSA. Dietrich Suck, Ralf Ficner, Uwe H. Sauer, Gunter Stier, EMBL, Meyerhostrasse 1, 69117 Heidelberg, Germany

The bifunctional protein pterin-4a-carbinolamine dehydratase (PCD) is a cytoplasmic enzyme involved in the regeneration of tetrahydrobiopterin, an essential cofactor of several monooxygenases. PCD is also found in cell nuclei forming a tight complex with the transcription factor HNF1. PCD binds to the dimerization domain of HNF1 and accordingly it is called dimerization cofactor of HNF1 (DCoH) as well. The functional enzyme PCD is a homotetramer while it interacts as a dimer with the dimeric HNF1.

The crystal structure of tetrameric PCD/DCoH from rat/human liver was solved by MIR and refined to a R-factor of 20.5% at 2.7 Å resolution (1). The single domain monomer (12 kDa) comprises three α -helices packed against one side of a fourstranded, antiparallel β -sheet. The homotetramer displays 222 symmetry and can be viewed as a dimer of dimers. In the dimer two monomers form an eight-stranded, antiparallel β -sheet with all helices packing against it on one side. In the tetramer the interface between both dimers is a central four helix bundle where each of the monomers contributes one helix to it. The concave, hydrophobic surface of the eightstranded β -sheet of the dimers is reminiscent of the saddle like shape seen in the TATA-box binding protein.

Recently, a bacterial homologue of PCD/DCoH, called PhhB, was found in *Pseudomonas aeruginosa* showing a dehydratase activity similar to the mammalian PCD. This prokaryotic PCD is also bifunctional, as it regulates the expression of the *P. aeruginosa* phenylalanine hydroxylase gene.

Here we present the overexpression, purification, and crystallization of the prokaryotic PCD. The crystal structure was solved by means of MAD using selenomethionine modified PCD and the refinement is currently in progress. The comparison of the mammalian PCD structure with the bacterial one, and preliminary results of mutational studies provide insight into the catalytic mechanism.

(1) Ficner, R., Sauer, U. H., Stier, G. and Suck, D. (1995) *EMBO J.* 14, 2034-2042.

PS04.01.115 CRYSTAL STRUCTURE OF ADP-RIBOSYL CYCLASE. C. D. Stout, G. Sridhar Prasad, E. A. Stura, D. E. McRee, Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, D. G. Levitt, H. C. Lee, Department of Physiology, University of Minnesota, Minneapolis, MN 55455

The crystal structure of ADP-ribosyl (ADPR) cyclase reveals a novel dimer in which the deep active site clefts of the monomers face toward the local two-fold axis. The monomers associate in such a way that a solvent filled tunnel connects the active sites. ADPR cyclase catalyses the synthesis of cyclic ADP-ribose (cADPR) from NAD in a reaction that requires displacement of nicotinamide followed by refolding of the nucleotide such that the N1 of adenine is covalently bonded to the C1' carbon of the terminal ribose with retention of configuration (1). The structure implies that the dual nature of the cyclase active sites is critical to carrying out this reaction. Soaking experiments coupled with mod-

eling of difference Fourier maps in progress may define the binding site of the substrate, intermediates or product. These results may infer an enzyme mechanism.

cADPR is emerging as an endogenous regulator of Ca^{2+} -induced Ca^{2+} release in cells (2). ADPR cyclase is abundant in *Aplysia* ovotestes and this source has been used for obtaining crystals (3). The enzyme was discovered in sea urchin eggs and is ubiquitous in tissues of marine invertebrates, amphibians, avians, and mammals, including humans (2). ADPR cyclase exhibits significant sequence homology to CD38, a lymphocyte differentiation antigen, which is a bifunctional ectozyme, also catalyzing the hydrolysis of cADPR.

The ADPR cyclase L-shaped monomer is comprised of a N-terminal helical domain and a C-terminal β -sheet containing domain resembling flavodoxin. There are 5 disulfides. The structure, and alignment of ADPR cyclase and CD38 sequences, suggests that the active site resides in the cleft between domains. Key residues for activity appear to be Trp77, Tyr81, His85, Thr96, Glu98, Asp99, Gly103, Tyr104, Asn107, Ser108 and Trp140. The structure was solved using a NCS averaged MIR map based on 6 derivatives. The current R-factor for all data in the range 8.0-2.4 Å is 0.22 (Rfree 0.31).

1. H. C. Lee *et al.*, *Nat. Struc. Biol.* 1, 143 (1994).
 2. H. C. Lee *et al.*, *Vitamins and Hormones* 48, 199 (1994).
 3. G. S. Prasad, *et al.*, *Proteins* 24, 138 (1996).
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PS04.01.116 STRUCTURAL STUDIES OF A BACTERIAL HELICASE. Helga Hoier, Dietmar Röleke, Cornelia Bartsch and Wolfram Saenger, Institut für Kristallographie, Freie Universität Berlin Takustr.6, 14195 Berlin, Germany

Helicase RepA is a typical helicase of the bacterial replication system. The enzyme unwinds double stranded DNA after binding to a flanking single stranded region. This process is fueled by ATP hydrolysis.

Single crystals of suitable size for x-ray crystallographic studies have been grown by the vapour diffusion method. They diffract to 2.8 Å resolution using synchrotron radiation. Space group was assigned to $P2_1$ with cell dimensions of $a=105$ Å, $b=180$ Å, $c=115$ Å, $\beta=95^\circ$. In agreement with electron microscopy studies we found that the protein is comprised of 6 identical 30 kDa subunits, forming a hexameric ring. The search for heavy atom derivatives is in progress.

PS04.01.117 THREE-DIMENSIONAL STRUCTURE OF O-ACETYL SERINE SULFHYDRYLASE FROM SALMONELLA TYPHIMURIUM. P. Burkhard*, E. Hohenester*, G.S.J. Rao#, P.F. Cook# and J.N. Jansonius*. *Department of Structural Biology, Biozentrum, University of Basel, Switzerland. #Department of Biochemistry, The University of Texas Southwestern Medical Center, Fort Worth, Texas, U.S.A

The A-isozyme of O-acetylserine sulihydrilase (OASS), an α -dimeric pyridoxal 5'-phosphate-dependent enzyme isolated from *Salmonella typhimurium* catalyses the synthesis of L-cysteine from O-acetyl-L-serine and sulfide. The pyridoxal form of the enzyme has been crystallized in the ortho-rhombic space group $P2_12_12_1$ with cell constants $a=54.3$ Å, $b=96.9$ Å and $c=144.4$ Å¹). The crystals diffract to 2.3 Å and contain one dimer per asymmetric unit. The subunit molecular weight is 34000.

The structure has been solved by MIRAS-phasing of six heavy atom derivatives and refinement is underway (current R-factor is 22% at 2.7 Å) OASS has a sequence similarity of about 30% to tryptophan synthase- β (TRPS β) but less than 20% of the residues are identical. Both enzymes have the same fold, but there are some major differences: The interface to the α - subunit in TRPS β

(residues $\beta 9$ - $\beta 22$ and $\beta 275$ - $\beta 295$) is missing in OASS. There are two additional surface helices in TRPS β (residues $\beta 23$ - $\beta 54$) and an additional loop ($\beta 260$ - $\beta 266$). 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 β 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 Patabhi 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 $a=47.07$, $b=53.82$, $c=77.7\text{Å}$. 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 Ca²⁺-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 C222₁, cell dimensions of $a=67.6$, $b=73.1$ and $c=156.6\text{Å}$, 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 CO₂ 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 CO₂ 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 105-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 Institute, 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.