The crystallographic R-factor of current model is 19.2% for 69,264 unique reflections with Fo > 2σF in the range of 8.0 - 2.3 Å. The root mean square deviations from ideal stereochemistry are 0.008Å for bond lengths and 1.095° for bond angles. The structural basis for the extreme thermostablity 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 ß-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 ß-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 ß-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 procaryotic PCD is also bifunctional, as it regulates the expression of the *P. areuginosa* phenylalanine hydroxylase gene.

Here we present the overexpression, purification, and crystallization of the procaryotic 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.

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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 modeling 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 ß-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, GlylO3, TyrlO4, AsnlO7, SerlO8 and Trpl40. The structure was solved using a NCS averaged MIR map based on 6 derivatives. The current R-factor for all data in the range 8.0-2.4Å is 0.22 (Rfree 0.31).

1. H. C. Lee *et al.*, Nat. Struc. Biol. **1**, 143 (1994).
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PS04.01.116 STRUCTURAL STUDIES OF A BACTERIAL HELICASE. Helga Hoier, Dietmar Röleke, Cornelia Bartsch and Wolfram Saenger, Institut fur 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, ß=95°. 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-ACETYLSERINE 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, Forth Worth, Texas, U.S.A

The A-isozyme of O-acetylserine sulihydrylase (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 Å[1]). 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-ß (TRPSß) 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 TRPSß