PS04.01.110 THE VERY HIGH RESOLUTION CRYSTAL STRUCTURE OF GLUCOSE OXIDASE FROM *P. AMAGASAKIENSE*. 1200 RESIDUES AT 1.79 Å RESOLUTION. Jörg Hendle[1], Hans-Jürgen Hecht[2], Henryk M. Kalisz[3] & Dietmar Schomburg[2]. [1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; Departments of [2]Molecular Structure Research and [3]Enzyme Technology, Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany

Glucose oxidase, a highly glycosylated flavo protein, is one of the most widely used enzymes in medical diagnostics and food processing. The enormous economic significance and intensive application of glucose oxidase as biosensor enzyme, as integral part of food production and in fermentation control was contradicted so far by the lack of structural knowledge.

The enzyme is a homodimer of 155 kDa with a glycan moiety of the 'high mannose' type representing 13% of the molecular weight. Crystals suitable for X-ray diffraction were obtained only after enzymatic deglycosylation removing 95% of the saccharide residues. As confirmed by the high resolution structure the monomer of the deglycosylated protein comprises 587 amino acid residues, 5 N-acetyl glucosamine, 3 mannose and 1 FAD molecule.

The exceptional stability of glucose oxidase might be based on the large surface area buried upon dimerization and defined by (i) hydrophobic contacts, (ii) extensive salt bridges and, surprisingly, (iii) by a cluster of well ordered water molecules trapped between the monomers with no contact to the bulk. The 67 kDa monomer is comprised of two domains only. The FAD binding domain is separated from the substrate binding domain by a deep cavity filled with a well defined network of 10 water molecules. At the given resolution this water cluster might suit as a starting point for the modeling of the substrate glucose bound to the binary enzyme:FAD complex.

The high resolution structure of glucose oxidase facilitates the use of protein engineering with the goal to design highly active enzyme derivatives with the capability to transfer directly electrons to semi-conductors and/or increased tolerance against hydrogen peroxide, sulfur dioxide and hydrogen sulfite.

PS04.01.111 STRUCTURE AND CRYSTAL PACKING STUDIES OF 4-OXALOCROTONATE TAUTOMERASE. Alexander B. Taylor*, Christian P. Whitman§, and Marvin L. Hackert* *Department of Chemistry & Biochemistry, The University of Texas at Austin, Austin, Texas 78712, §Medicinal Chemistry Division, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712

4-Oxalocrotonate tautomerase (4-OT) is a highly efficient enzyme with an unusual mode of catalysis and an unexpected crystal packing scheme. 4-OT takes part in the meta-fission pathway encoded by the *Pseudomonas putida mt-2* TOL plasmid pWW0 for catabolism of toluene, *m*- and *p*-xylene, 3-ethyltoluene, and 1,2,4-trimethylbenzene to Krebs cycle intermediates.[1] 4-OT catalyzes the isomerization of 2-oxo-4-hexenedioate to 2-oxo-3-hexenedioate through the intermediate, 2-hydroxymuconate. The structure has been solved to 2.5Å resolution by the Molecular Replacement method with a recombinant 4-OT isozyme from *Pseudomonas* sp. Strain CF600 serving as a model.

4-OT is a hexamer composed of small monomers of 62 amino acids arranged with 32 symmetry.[2] The monomer consists of a two-stranded parallel β-sheet with a linking helix; dimerization leads to a four-stranded sheet with antiparallel helices on one side. Each dimer contains two active sites with an unusual catalytic amino-terminal proline.[3] The active site has residues contributed from both subunits of the dimer.

4-OT crystallizes in space groups R3 (a=98.7Å, α=52.5°, 6 dimers/a.u.) and P321 (a=88.0Å, c=124.6Å, 4.5 dimers/a.u.). In both crystal systems, the crystal packing is dictated by a shifted stack of three hexamers. Current work includes cocrystallization of native enzyme with mechanism-based inhibitors.

[1]Chen L. et al. (1992) *JBC*. **267**, 17716-17721.
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[3]Stivers, J.T. et al. (1996) *Biochemistry*. **35**, 803-813.

PS04.01.112 PRELIMINARY CRYSTALLOGRAPHIC STUDY OF FORMALDEHYDE DISMUTASE. Akihito Yamano[1], Tsuneyuki Higashi[1], Hideshi Yanase[2], Nobuo Kato[3], Hideaki Moriyama[4], Nobuo Tanaka[4] & Yukiteru Katsube[1], [1]X-ray Research Laboratory, Rigaku Corp., Tokyo, Japan, [2]Department of Biotechnology, Faculty of Engineering, Tottori University, Tottori, Japan, [3]Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto, Japan, [4]Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Kanagawa, Japan.

Formaldehyde dismutase (FDM) (MW=180kDa) was crystallized by the hanging drop vapor diffusion technique. FDM, found in a formaldehyde resistant bacterium (Pseudomonas putida), catalyzes the dismutation of aldehydes and alcohol. Amino acid sequence alignment indicates that FDM has the NAD-binding motif conserved among the NAD-dependent dehydrogenases. However, the binding mode of NAD(H) in FDM appears to be very different from those in other NAD-dependent dehydrogenases. The NAD(H) in FDM does not dissociate but stays bound in the enzyme throughout the reaction, therefore there is no enhancement of its activity on the addition of an excess amount of NAD(H). In order to elucidate the nature of the stronger binding, we started a project to determine the three-dimensional structure of FDM.

Crystals suitable for an X-ray diffraction experiment were acquired by equilibration of FDM solution (17.5 mg/ml) against 30%(wt/v) AS in 100mM potassium phosphate buffer (pH7.0). The protein solution was filtered to eliminate unwanted aggregation detected by a DynaPro (Protein Solutions) prior to the crystallization. FDM crystallized in the tetragonal space group $P4_1$ (or $P4_3$) with unit cell dimensions a=b=92.4Å and c=225.0Å. X-ray diffraction data were collected to 3Å resolution on an RAXIS IIc. We are now trying MR methods using coordinates from NAD-dependent dehydrogenase structures.

PS04.01.113 CRYSTAL STRUCTURE OF XYLOSE ISOMERASE FROM THERMOPHILIC BACTERIA THERMUS CALDOPHILUS. Changsoo Chang, Byung Chul Park*, Dae-Sil Lee*, and Se Won Suh, Department of Chemistry and Center for Molecular Catalysis, Seoul National University, Seoul 151742, Korea, *Korea Research Institute of Bioscience and Biotechnology, KIST, P.O.Box 115 Korea

Xylose isomerase catalyzes the conversion of D-xylose to D-xylulose (D-glucose to D-fructose) and divalent cations (Mn^{++}, Co^{++}, or Mg^{++}) are required for activity. Several crystal structures of xylose isomerases have been determined and there is a great interest in protein engineering of this enzyme. *Thermus caldophilus* xylose isomerase is a tetrameric enzyme with monomer molecular weight of 43,000 Da. The optimum temperature of *Thermus caldophilus* xylose isomerase is 93 °C, and this enzyme is stable up to 95 °C.

The crystal structure of xylose ismomerase from extreme thermophile, *Thermus caldophilus,* has been solved by molecular replacement using xylose isomerase from *Actinoplanes missouriensis* (Jenkins. *et al.* (1992) Biochem. **31**, 5449 - 5458 PDB ID code 3xin) as a starting model.

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.

(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 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).
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 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