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.
[2]Subramanya, H.S. et al. (1996) *Biochemistry*. **35**, 792-802.
[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.