

**PS04.01.64 THE STRUCTURE OF THE DETOXIFICATION ENZYME: GLUTATHIONE S-TRANSFERASE D21 FROM *DROSOPHILA MELANOGASTER*.** Ping-lin Ong<sup>1,2</sup>, John P. Rose<sup>2</sup>, C.-P. D. Tu<sup>3</sup> and B.C. Wang<sup>2</sup>. <sup>1</sup>Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260, U.S.A., <sup>2</sup>Dept. of Biochemistry and Molecular Biology, Univ. of Georgia, Athens, GA 30602, U.S.A. and <sup>3</sup>Dept. of Biochemistry and Molecular Biology, Pennsylvania State Univ., College Park, PA 16802, U.S.A.

The crystal structure of *Drosophila melanogaster* Glutathione S-transferase D21 (GST-D21) has been determined at 2.5Å resolution by molecular replacement.

Glutathione S-transferases (GSTs) (EC 2.5.1.18) are a family of multi-functional enzymes involved in the cellular detoxification and excretion of many physiological and xenobiotic substances. Five GST classes: mu, pi, alpha, theta, and microsomal have been reported.

GST-D21 is a member of the theta class GSTs. It was crystallized from PEG4000, pH 7.5. A data set to 2.5Å resolution was collected on a Siemens X-100 area detector. The data were indexed integrated and scaled using XENGEN 2.1 and gave an R<sub>sym</sub> of 5.3%. The crystal indexed in a primitive monoclinic lattice with cell constants a=53.76Å, b=89.70Å, c=50.89Å, β=113.75°. There are two GST-D21 molecules in the asymmetric unit and the Matthews coefficient is calculated to be 2.33. Analysis of the systematic absences uniquely determined the P2<sub>1</sub> space group.

The structure was solved by molecular replacement (AMoRe) using blow fly GST (Wilce, *et al.*, 1995) as a search model. The initial model was rebuilt by fitting correct sequence into the 2Fo-Fc electron density map. The structure was refined against 8-2.5Å data using X-PLOR 3.1. Non-crystallographic symmetry constraints were imposed throughout the refinement.

The structure at the current stage of refinement, 3476 non-hydrogen atoms, no solvent molecules are included, has an R value of 22% and an R<sub>free</sub> value of 29%. A structural comparison of the different GST classes and a structural explanation of GST function will be described in detail.

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**PS04.01.65 THE HUMAN PI-CLASS GLUTATHIONE S-TRANSFERASE: A TARGET FOR STRUCTURE-BASED DRUG DESIGN.** Aaron J. Oakley<sup>1</sup>, Jamie Rossjohn<sup>1</sup>, Mario Lo Bello<sup>2</sup> and Michael W. Parker<sup>1</sup>. <sup>1</sup>The Ian Potter Foundation Protein Crystallography Laboratory, St. Vincent's Institute of Medical Research, Fitzroy 3065, Victoria, Australia. <sup>2</sup>Department of Biology, 2nd University of Rome, "Tor Vergata", Rome, Italy

Glutathione S-transferases (GSTs) are a family of enzymes that conjugate electrophilic substrates to the tripeptide glutathione (GSH, γ-Glu-Cys-Gly). In humans they are involved in the development of resistance of abnormal cells towards anti-cancer drugs through their GSH-conjugating activity. There are four main classes of cytosolic GSTs in mammals: alpha, mu, pi and theta. The pi-class GST is considered to be particularly important and is expressed in a diverse range of tumours. The human pi-class GST (HPGST) is therefore of interest as a target for structure-based drug design: potent inhibitors could be used in conjunction with chemotherapy to enhance the efficacy of the treatment.

Important for structure-based drug design is the investigation of protein structure with existing inhibitors and substrates. Ethacrynic acid (EA) has been used in clinical trials as a GST inhibitor, however the HPGST is particularly efficient at conjugating it to GSH. The determination of the structure of the HPGST in complex with EA and its glutathione conjugate is reported. We have also solved the structure of the Y108F HPGST mutant. These structures suggest a mechanism by which the enzyme catalyses the addition of EA to GSH.

Until recently, only low resolution inhibitor structures of the human pi-class GST have been studied. We now report the determination of the structure of HPGST in a novel space group (C2) at 1.9Å resolution. A number of other HPGST-inhibitor complexes have also been solved.

**PS04.01.66 PRELIMINARY X-RAY STRUCTURE ANALYSIS OF RAB GERANYLGERANYL TRANSFERASE.** Hong Zhang, Miguel C. Seabra\* and Johann Deisenhofer Howard Hughes Medical Institute and \*Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Rab proteins comprise a family of small GTPase that play a key role in the regulation of vesicular membrane traffic. The attachment of a 20 carbon isoprenoid, geranylgeranyl, to the C-terminal cysteine motifs of Rabs is crucial for their membrane association and function. This post-translational modification is a complex reaction that requires a catalytic Rab geranylgeranyl transferase (Rate GGTase) and a Rab escort protein (REP).

The Rab GGTase is a heterodimer consists of two tightly associated α and β subunits of 65 and 37kD, respectively. Both subunits of GGTase have been cloned and co-expressed in the baculovirus/Sf9 insect cell system. The whole protein has been purified to homogeneity. Diffraction quality crystals have been obtained from buffered PEG solution using the vapor diffusion method. The crystals diffract to about 2.8Å resolution and they are of P1 space group with unit cell dimensions a=57.96Å, b=77.47Å, c=123.54Å, α=74.89°, β=80.39° and γ=67.98°. We will present the recent progress in our attempt to determine the three dimensional structure of Rab GGTase.

**PS04.01.67 HIGH RESOLUTION STRUCTURE OF HUMAN ORNITHINE AMINOTRANSFERASE (OAT) COMPLEXED WITH alpha-KETOGLUTARATE: IMPLICATIONS OF THE REACTION MECHANISM.** Betty W. Shen, Sapan A. Shah, and Axel T. Brunger. Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA

The crystal structure of an OAT/alpha-ketoglutarate complex is refined against a 2.0Å resolution data set collected at -150°C using a CCD detector at CHESS with synchrotron radiation of 0.92Å wavelength. The data is 99.6% complete with R<sub>sym</sub> = 7.2% (25.4%). The unit cell dimensions of the frozen crystal are slightly smaller than the data set collected at ambient temperature, which was used for solving the structure of OAT (Shen *et al.*, manuscript in preparation). The refined coordinates at 2.5Å resolution was used directly for the refinement of the enzyme-substrate complex. A strong, continuous tube of electron density in the partially refined density map of all three protomers in the asymmetric unit suggested the presence of a molecule of alpha-ketoglutarate in the active site pocket. The presence of the substrate molecules is further confirmed by its persistence in a simulated-annealing omit map.

Interestingly, the putative alpha-ketoglutarate molecule occupies a site physically remote from the proposed binding site for ornithine. Since the structure and charge distribution of the two substrates are largely different, it is conceivable that the substrates of the two half reactions are bound to different residues in separate locations. This hypothesis is consistent with previous reports that the two half reactions displayed different pH optima and that the km of OAT for ornithine and alpha-ketoglutarate followed entirely different pH dependencies.