PS04.01.68 THREE DIMENSIONAL STRUCTURE OF HUMAN ORNITHINE AMINOTRANSFERASE: CHARGE DISTRIBUTION AT THE ACTIVE SITE EXPLAINS THE SELECTIVITY FOR W - GROUP OF DIBASIC AMINO ACIDS. Betty W. Shen*, Michael Hennig#, Erhard Hohenester#, and Johan N. Jansonius#.*Department of Molecular of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT USA; #Biozentrum, University of Basel, Basel CH4056 Switzerland

Ornithine aminotransferase (OAT) catalyses exclusively the transfer of the delta-amino group of ornithine to alpha-ketoglutarate, forming glutamate-r-semialdyhyde and glutamic acid. It is one of the major matabolic enzymes that modulate nitrogen fixation in the urea cycle as well as the synthesis of argine, proline, and spermidine.

Inhibition of OAT has recently been explored as means of detoxification in animal experiments. In view of its diversified roles, it is of interest to compare the structure of OAT with other subgroups of the transaminase superfamily in order to understand the basis of its extraordinary specificity.

The structure of OAT is presented at 2.5Å resolution. The recombinant enzyme crystallized in the trigonal space group P3(2)21 with one and a half dimers per asymmetric unit. The refined model has a R-factor of 17.9% (free-R = 23.3%) and excellent stereochemistry. Each subunit of the dimeric enzyme contains three distinct regions: an extended N-terminal segment, a cofactor binding large domain, and a relatively flexible small domain. The OAT dimer is stabilized by a large contact surface between the large domains of the two subunits and between the N-terminal segment of one sununit with the large domian of the other. The pyridoxal-5'-phosphate (PLP) cofactor is bound to the large domain close to the subunit interface and is stabilized through multiple hydrophobic and electrostatic interactions to both subunits. Glu235 and Arg413, which form a salt bridge in at the entrance to the active site pocket, are the most probable residues for the binding of ornithine. The location of Glu235 explains the selectivity of OAT for the delta-amino group despite the presence of the more reactive alpha-amino groups of ornithine and glutamate.

PS04.01.69 CRYSTALLOGRAPHIC STUDY OF BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE FROM ESCHERICHIA COLI. Kengo Okada*, Ken Hirotsu*, Hidoyuki Hayashi** and Hiroyuki Kagamiyama**, Department of Chemistry, Faculty of Science, Osaka City University*, Department of Biochemistry, Osaka Medical College**

Branched-chain amino acid aminotransferase from *Escherichia coli* [E.C.2.6.1.42] (BCAT) is one of vitamin B_6 enzyme having pyridoxal-5'phosphate (PLP) as a co-factor. BCAT forms a hexamer (M.W. 204,000 Da) of six identical subunits each consisting of 308 residues (M.W. 34,000 Da). Crystallization of BCAT was performed by hanging-drop vapor diffusion method. Two polymorphic crystals with good quality for X-ray crystallography were obtained and diffracted to 2.2Å resolution with a conventional X-ray source. The crystals belong to the orthorhombic space group $C222_1$ with unit cell dimensions of a=156.0, b=101.2, c=141.3 Å, and the monoclinic space group $C2$ with unit cell dimensions of a=135.3, b=144.1, c=102.9 Å, β=136.1°, respectively. Each Matthews' Vm value is 2.7 ($C222_1$) and 3.4 ($C2$), which indicates three subunits related by a three-fold noncrystallographic axis in the asymmetric unit. Multiple isomorphous replacement (MIR) techniques were used to determine the three-dimensional structure of BCAT. We succeeded in preparing two kinds of Hg derivatives (EMTS and CH_3CH_2HgCl), and initial phasing at 3.0 Å resolution.

PS04.01.70 STRUCTURE AND FUNCTION OF GLYCINE METHYLTRANSFERASE FROM RAT LIVER. Zhuji Fu, Yongbo Hu, Fusao Takusagawa, Departments of Chemistry and Biochemistry, University of Kansas, Lawrence, KS 66045-0045, Kiyoshi Konishi, Yoshimi Takata and Motoji Fujioka, Department of Biochemistry, Toyama Medical and Pharmaccutical University, Faculty of Medicine, Sugitani, Toyama 930-01, Japan

Biological methylation reactions utilizing S-adenosylmethionine (AdoMet) as the methyl donor are widespread in nature, and participate in a wide variety of cellular processes through methylation of nucleic acids, proteins, phospholipids, and small molecules. Glycine methyltransferase (S-adenosyl-L-methionine:glycine methyltransferase, EC 2.1.1.20; GMT) catalyzes the AdoMet-dependent methylation of glycine to form sarcosine. GMT, unlike most AdoMet-dependent methyltransferases, is an oligomeric protein consisting of four identical subunits.

The enzyme was crystallized by the hanging drop vapor diffusion method. The crystal belongs to the orthorhombic space group $P2_12_12$, with unit cell dimensions of a= 86.4, b= 175.7, c= 45.5 Å and with two subunits in the asymmetric unit. The crystal structure has been determined at 2.4 Å resolution by MIR method. The current crystallographic R-factor is 0.19 for all data. In the structure, the N-terminal regions (1-20 residues) are far from the subunit and go into the opposite subunit. Furthermore, Glu15 is involved in formation of the active site of the opposite subunit. The detailed active site geometry and the AdoMet binding scheme will be described along with the catalytic mechanism of GMT at the meeting.

PS04.01.71 ENZYME ACTIVITY AT 100°C: CITRATE SYNTHASE FROM *PYROCOCCUS FURIOSUS* Rupert JM Russell, David W Hough, Michael J Danson and Garry L Taylor.School of Biology and Biochemistry,University of Bath, BA2 7AY, U.K.

The ability of enzymes from hyperthermophiles (growth temperature > 85°C) to be stable and optimally active at high temperatures has widespread biotechnological applications. In order to gain insights into enzyme mechanism at 100°C, the crystal structure of the closed form (liganded) of citrate synthase from the hyperthermophilic Archaeon *Pyrococcus furiosus* has been determined to 3.0.Å This has allowed direct structural comparisons between the same enzyme but from hosts which show different temperature optimum for citrate synthase activity: 37°C (pig), 55°C (*Thermoplasma acidophilum*) and now 100°C (*P. furiosus*). Pig citrate synthase undergoes a large conformational change upon substrate binding, but the structures of the thermophilic citrate synthases suggest that the magnitude of the conformational change may not be a constant feature of this enzyme mechanism. Coenzyme A is recognised in a slightly different manner from that observed in the pig enzyme. The differences in the active site and magnitude of the conformational change between the enzymes will be rationalised on the basis of performing the same catalytic mechanism but at different temperatures.