PS04.01.72 CRYSTAL STRUCTURE OF ASPARAGINE SYNTHETASE FROM *ESCHERICHIA COLI*. Toru Nakatsu, Hiroaki Kato and Jun'ichi Oda. Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

Three dimensional structure of asparagine synthetase[L-aspartate: ammonia ligase (AMP-forming) EC 6.3.1.1] from *Escherichia coli* which catalyzes the ligation of aspartic acid and ammonia in the aid of MgATP has been determined using its cysteine-free mutant by multiple isomorphous replacement at 2.7 Å resolution. Overall structure of asparagine synthetase is almost similar to that of catalytic domain of aspartyl-tRNA synthetase from yeast. Despite their low seaquence similarity, the structure-based alignment shows interesting features that a number of catalytically important residues are conserved in entire region. Mechanistically the reaction of both synthetases proceed through an aspartyl-adenylate intermediate although each synthetase utilizes the different carboxyl group, α- or β- carboxylate of aspartic acid.

The asparagine synthetase is a homodimer and its subunit contains of 330 amino acid residues (Mr 37kDa). The replacement of Cys51 and Cys315 with Ala residues was necessary to get suitable crystals for three dimensional structure determination. The crystals belong to the spacegroup $P2_1$ with cell constant a = 52.9Å, b = 126.2 Å, c = 52.8 Å, β = 105.3°. The M.I.R. phase was determmined using Sm and Pt derivatives and was improved by solvent flattening and non crystallographic symmetry averaging. Current model includes 327 residues and the R factor is 19.5 % at 10.0 - 2.7 Å resolution. The asparagine synthetase structure consists of a six-stranded anti-parallel β-sheet sandwiched with α-helices. This topology is same as that of aspartyl-tRNA synthetase with r.m.s.d. of 1.9 Å for 217 Cα positions.

Further elucidation of the product bound form is in progress to allow the determination of the binding residue, and of similarities of the catalytic mechanism.

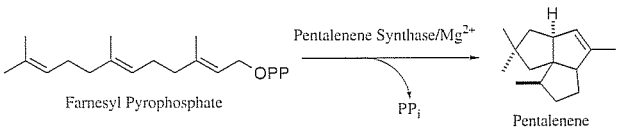
PS04.01.73 INHIBITORS OF GLUTAMINE SYNTHETASE FROM *SALMONELLA TYPHIMURIUM* AND *MYCOBACTERIUM TUBERCULOSIS*. Harindarpal Gill, Gunter Harth, Marcus Horwitz, David Eisenberg, Molecular Biology Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles. Los Angeles, California, 90095-1570, USA

Tuberculosis has re-emerged as a global health concern— causing 8 million new cases of pulmonary tuberculosis and 3 million deaths annually. Existing drugs are becoming less effective because of a rise in anti-*Mycobacterial* resistance. Discovery of novel drug targets are complicated by the need to penetrate the thick cellular envelope of *Mycobacteria.* However, recently it has been discovered that pathogenic *Mycobacteria (tuberculosis and bovis)* secrete enzymes into their immediate environment. In particular, Glutamine Synthetase (GS) is secreted into the medium where it is thought to play a role in the synthesis of poly-(L-glutamine-L-glutamate) chains which are a constituent of pathogenic *Mycobacteria* cell walls.

This secreted GS is our primary target for drug-design. Bacterial GS is a dodecamer with 12 separate active sites, one each between a pair of subunits. In contrast, eukaryotic GS is an octamer implying that the pockets that form each active site in human GS must be significantly different than bacterial GS, thus allowing for drug-specificity. Two inhibitors— methionine sulfoximine (MetSox) and phosphinothricin (PPT)— are potent inhibitors of GS. Preliminary results from these inhibitors demonstrate a static inhibition on the *Mycobacterium tuberculosis* (TB) growth curve. Efforts are underway to co-crystallize these inhibitors with GS from *Salmonella typhinurium* in hope to obtain a high resolution structure which might serve as a better model for computational drug- design. Future studies of this kind are planned with TB-GS and other studies involving fully-adenylylated GS are being pursued.

PS04.01.74 PRELIMINARY CRYSTALLIZATION AND DIFFRACTION ANALYSIS OF RECOMBINANT PENTALENENE SYNTHASE. Charles A. Lesburg, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 USA

A sesquiterpene cyclase found in *Streptomyces* UC5319, pentalenene synthase, has been crystallized in space group P63 with unit cell dimensions a = b = 183.5 Å, and c = 56.5 Å. Hexagonal prismatic crystals, approximately 0.2 x 0.2 x 0.3 mm, diffract to approximately 2.9 Å resolution using monochromatic synchrotron radiation and to 3.5 Å resolution using CuKα radiation. Pentalenene synthase catalyzes the biosynthesis of pentalenene, a precursor to the pentalenolactone family of antibiotics. From the universal (and achiral) building block, farnesyl pyrophosphate, pentalenene synthase catalyzes the formation of four stereocenters in the construction of the three fused five-membered rings of pentalenene.



PS04.01.75 STRUCTURES OF ELECTRON TRANSFER FLAVOPROTEIN FROM HUMAN AND *PARACOCCUS DENITRIFICANS*. David L. Roberts, Frank E. Frerman§, and Jung-Ja P. Kim, Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226, §University of Colorado Health Science Center, Denver Colorado, 80262.

Mammalian electron transfer flavoprotein (ETF) contains a single equivalent of FAD per αβ dimer, and functions as an electron shuttle between the primary dehydrogenases that are involved in fatty acid and amino acid metabolism and the membrane bound electron transfer flavoprotein dehydrogenase (ETF-QO). ETF from *Paracoccus denitrificans* has been shown to be highly homologous with the human enzyme, showing > 55% amino acid sequence identity.

The structure of the human ETF protein has been solved to 2.1 Å. The enzyme crystallizes in the monoclinic space group $P2_1$, with unit cell parameters a=47.46, b=104.92, c=63.79 Å, and ß=110.09°. The phases were solved using MIR, with three heavy atom derivatives. Density modification procedures including solvent flattening and phase combination with SigmaA were used to improve the initial phases. The refinement was carried out using XPLOR, with alternating rounds of manual adjustment of the model. The final R factor without added waters is 23.3%, with R_{free}=30.3% for all reflections between 15-2.1 Å.

Using this refined human ETF model, molecular replacement was used to solve the *P. denitrificans* ETF structure to 2.5Å. The *P. denitrificans* ETF crystallizes in the orthorhombic space group $P2_12_12_1$, with unit cell parameters a=70.52, b=80.13, and c=184.00 Å. After initial refinement, the R-factor is 27.5%, with R_{free}=38.5%. We are presently refining the model.

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