

## 03-Crystallography of Biological Macromolecules

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$\omega$ -Amino acid:pyruvate aminotransferase( $\omega$ -APT) from *Pseudomonas* sp. F126 is a Pyridoxal 5'-phosphate(PLP) dependent enzyme. This enzyme catalyzes the reversible transamination of the  $\omega$ -amino group from  $\beta$ -alanine to pyruvate and produces malonic semialdehyde and L-alanine. The structure of this enzyme was solved at 2.0Å resolution using MIRA method and was refined with 1.8Å resolution data(N. Watanabe et al, Acta Cryst. A suppl., 1990, C19). In order to determine the mechanism, crystals of the enzyme complex with various substrates were prepared by cocrystallization and solved structures were compared.

For each data set, intensity data greater than 1.8Å resolution was collected using synchrotron radiation at the BL-6A2 station, PF, with a Weissenberg camera and Fuji Imaging Plate(N. Sakabe, Nucl. Instrum. Method, 1991, A303, 448-463). Data were processed using the "WEIS" program system(Higashi, T., *J. Appl. Cryst.*, 1989, 22, 9-18). With the L-alanine/ $\omega$ -APT cocrystals, data to 1.4Å resolution was collected yielding 76,761 unique reflections with a R-merge(I) of 5.64%. The structure was extensively refined using PROLSQ with 8.0-1.6Å data to a R-value of 15.5%. Difference fourier maps were calculated and the substrate model was built in. This result shows that L-alanine and PLP form a Schiff base, indicating a radically different reaction mechanism of  $\omega$ -APT from aspartate aminotransferase. This interpretation is supported by other amino donor substrate complexes collected and refined to 1.8Å resolution.

A detailed comparison among the liganded and unliganded  $\omega$ -APT structures has been carried out. There are no large conformational change in the structure of the protein but there are major changes of solvent structure around the ligand binding region.

The substrate side chains are classified into three groups; linear, branch and aromatic. The regularities of side chain stacking will be discussed with respect to the hydrogen bond and hydrophobic interactions.

**PS-03.05.31** CRYSTAL STRUCTURE OF D-GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM *PALINURUS VERSICOLOR* AT 1.8Å RESOLUTION BY Shi-ying Song\*, Jun Li, Zheng-jiong Lin and Chen-lu Tsou  
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D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in glycolysis and shows cooperativity in NAD<sup>+</sup> binding and half-of-the-sites properties toward reaction with some thiol modification reagents.

The enzyme was extracted from the tail muscle of South China sea Lobster *P.versicolor* and crystallizes in a form different from that for the Atlantic lobster *H.americanus* enzyme. The space group is C2 with cell dimensions a=128.11Å, b=99.61Å, c=80.69Å and  $\beta$ =114.4° with half of the molecule per asym. unit[Song, et al., (1983), *J.Mol.Biol.* 171, 225-228]. The structure was determined by the molecular replacement method using the atomic coordinates of the known structure of *H.americanus* solved by Rossmann's group and 2.7Å 4-circle diffractometer data.

The high resolution data was collected by synchrotron radiation-Fuji imaging plate-Sakabe's Weissenberg camera system at BL6A2 of photon factory in KEK. The whole data set contains 172806 reflections (55850 unique reflections) with R-merge of 5.77%. The structure refinement was carried out using the programs XPLOR and PROLSQ and model building techniques on PS 390 based on the 2.7Å model. The current model containing 2 NAD<sup>+</sup> molecules, 2 sulphate ions and 153 ordered water molecules gives a crystallographic R-factor of 0.216 for 50031 reflections with F>2.5 r within 5.0-1.8 Å resolution and a stereochemistry with r.m.s. deviations from ideal geometry of 0.018Å for bond lengths and 3.42° for bond angles.

The folding of subunit resembles closely the known structure of *H.americanus* GAPDH and *B.stearothermophilus* GAPDH. The structure similarity between *B.stearothermophilus* GAPDH and *P.versicolor* GAPDH in catalytic domain is higher than that in NAD<sup>+</sup>-binding domain. The conformation of the adenine about the glycosidic bond is anti for the ribose ring not only in the red subunit but also in the green subunit. The r.m.s. differences in atomic positions between the green and red subunits are 0.39Å for the C<sup>α</sup> atoms and 1.27Å if other atoms are included, showing the existence of minor side chain asymmetry. Detailed analysis of the structure after further refinement will be presented in the Congress.

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**PS-03.05.32** THE CRYSTAL STRUCTURE OF HMG-CoA REDUCTASE FROM PSEUDOMONAS MEVALONII. By C. Martin Lawrence<sup>1</sup>, Victor W. Rodwell<sup>2</sup> and Cynthia V. Stauffacher<sup>1\*</sup>, <sup>1</sup>Department of Biological Sciences, <sup>2</sup>Department of Biochemistry, Purdue University, West Lafayette, IN 47907

*Pseudomonas mevalonii* HMG-CoA reductase is a four electron oxidoreductase that catalyzes the interconversion of HMG-CoA and mevalonate, the first committed step in polyisoprenoid biosynthesis. In mammals this reaction is the rate-limiting step in the synthesis of cholesterol and the enzyme is the target of anti-cholesterol drugs. We have crystallized HMG-CoA reductase in the cubic space group I4<sub>1</sub>32, a=229.4 Å, with two monomers (45 kD/monomer) per asymmetric unit. Native and derivative data sets have been collected to 2.8 Å resolution on a Xuong-Hamlin area detector. Gold and mercury derivatives were used to produce a 3.0 Å MIR map in which clear secondary structure was evident. The phases were then