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ω-Amino acid:pyruvate aminotransferase(ω-APT) from *Pseudomonas* sp. F126 is a Pyridoxal 5'-phosphate(PLP) dependent enzyme. This enzyme catalyzes the reversible transamination of the ω-amino group from β-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. Apple. Cryst.*, 1989, **22**, 9-18). With the L-alanine/ω-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 Shiff base, indicating a radically different reaction mechanism of ω-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 ω-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 National Laboratory of Biomacromolecules, Institute of Biophysics, Beijing, China

D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in glycolysis and shows cooperativity in NAD^+ 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 ß=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 diffractomator 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^+ 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^+-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^a 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.
We thank Prof. M.G.Rossmann for his encouragement and valuable suggestions. Thanks also go to Prof. N. Sakabe for his important help in data collection.

PS–03.05.32 THE CRYSTAL STRUCTURE OF HMG-CoA REDUCTASE FROM PSEUDOMONAS MEVALONII. By C. Martin Lawrence[1], Victor W. Rodwell[2] and Cynthia V. Stauffacher[1]*, [1]Department of Biological Sciences, [2]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 $I4_132$, 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

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improved with the use of solvent flattening and averaging over the twofold non crystallographic symmetry and the sequence fit to the resulting electron density. A single data set from selenomethionine HMG-CoA reductase was also collected. Difference Fouriers clearly showed the methionine positions, confirming the chain tracing. The HMG-CoA reductase monomer is composed of two mixed α/β domains. The large domain contains both the amino and carboxy terminal ends, while the small domain is composed of an internal 100 residues. The large domain structure shows a central 24 residue α-helix surrounded on three sides by all α-helical, mixed β/α and β-sheet walls. The small domain is composed of 4 anti-parallel β-strands and two connecting α-helices. Two HMG-CoA reductase monomers form a tightly bound dimer. The core of the dimer is formed by the helical structure of the large domain, including a four helix bundle centered on the dimer axis. Two active site clefts are found at the dimer interfaces with residues from each monomer involved in the formation of the two active sites. Catalytically important residues identified by site-directed mutagenesis line the clefts. Difference Fouriers from native crystals soaked in substrates show that HMG-CoA is bound by the large domain while NADH is bound by the small domain.

PS-03.05.33 The study of Cu(I) and Cu(II) Dopamine β Hydroxylase (DBH) model complexes by X-ray absorption and diffraction

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DBH, a tetrameric glycoprotein of 603 amino acids per monomer, is a copper enzyme which catalyses the transformation of Dopamine into Noradrenaline. According to the hydroxylation reaction mechanism proposed by Klinmann (Brenner, Murray & Klinmann,1986) two copper atoms are involved and play different roles: one is used as electron transfer while the second one, located near the substrate fixation site, directly acts on the dopamine hydroxylation via a CuOOH species. Recently EXAFS studies on the DBH active site brought new insights on the active site by revealing the presence of a S-methionine ligand coordinated to Cu(I), not present in the oxidized Cu(II) form of DBH (Scott, Sullivan, De Wolfe, Dolle, Kruse, 1988; Pettingall, Strange & Blackburn, 1991; Blackburn, 1993).

Several approaches of the study of the DBH structure have been developped in our laboratory. A series of Cu-complexes which are supposed to mimic the structure and the properties of the DBH active site and including N, O and S atoms have been prepared.

X-ray Absorption of nine Cu(I) and Cu(II) model complexes has been measured on XAS II station at LURE (Orsay-Paris). EXAFS and XANES spectra (Fig. 1) exhibit differences in accordance with the Cu ligation atom nature (N, O, S) and the Cu electronic state.

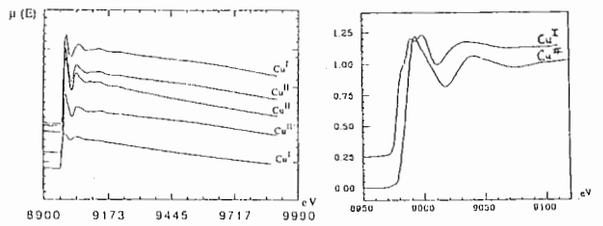

Fig. 1 - EXAFS and XANES experimental spectra of Cu(II) and Cu(I) complexes

The interpretation of our results is in progress and is based on the crystallographic structure obtained for two complexes of this series : an example is given on Fig. 2.

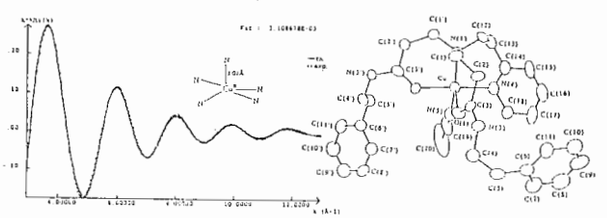

Fig.2 - EXAFS simulation and fit to the experience

The molecular simulation of our models with different force fields (MM2, GROMOS, AMBER) is also used to predict their conformation with some confidentiality with regard to the X-ray diffraction and absorption data.

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PS-03.05.34 CRYSTAL STRUCTURE OF THE MONOFUNCTIONAL CHORISMATE MUTASE FROM *BACILLUS SUBTILIS*

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Chorismate mutase (CMase) is an enzyme in the pathway leading to biosynthesis of aromatic amino acids (shikimate pathway). It is the only characterized example of an enzyme in primary metabolism that catalyzes a pericyclic process, the rearrangement of chorismate to prephenate. So far, the mechanistic details of this enzyme-catalyzed reaction remain a mystery. The simplicity of this rearrangement and the existence of a readily occurring uncatalyzed rearrangement should provide much insight for understanding enzyme-mediated rate-acceleration. Furthermore, catalytic antibodies with chorismate mutase activity have been prepared (Hilvert *et al.*,1988 and Jackson *et al.*,1988) and comparison of the active sites in the enzyme and antibodies will be a novel study to understand catalysis.

CMase from *Bacillus subtilis*, Mr=14.5kD, is the smallest characterized natural chorismate mutase. The size of the enzyme, its Michaelis-Menten kinetics and absence of other associated enzyme activites (Gray *et al.*,1990) result in a simpler experimental system than other bifunctional and more complex chorismate mutases (eg. from *E.coli* and yeast) for structural analysis to understand catalysis. This enzyme crystallizes in space group $P2_1$, with 12 monomers in the asymmetric unit. The crystal structure of CMase was determined using multiple isomorphous replacement method (derivatives - Hg, Ir, Os and a Seleno-methionine protein with 72 Seleniums in the asymmetric unit!). The figure of merit is 0.70 for reflections at 15.0-3.5Å. A polyalanine trace of approximately 76 residues per monomer was made using the MIR map. This constitutes all secondary structure elements and 60% of the asymmetric unit. One round of phase combination using this partial polyalanine model improved the quality of the electron density significantly, especially the side chains and loop regions. The trace of the structure to 2.7Å is almost (87%) complete. Upon completion of the trace, phase extension and refinement will be done to 1.9Å.

Data of complexes with a transition state analog and with the product, prephenate, are also available. Difference fourier maps have revealed the location of the active site. The structural analysis of the active site should provide some long-awaited for mechanistic information on the catalysis by chorismate mutase.

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