

03-Crystallography of Biological Macromolecules

on a rotating anode (CuK α) generator with a merging R of 9.9% (for 98793 measurements of 19667 reflections with $I > 4\sigma$). The sheep 6-PGDH structure has been solved and refined to 2.5Å resolution (Adams, M.J., Gover, S., Leaback, R., Phillips, C. & Somers, D.O'N. *Acta Cryst.* B47, 817-820) allowing the possible elucidation of the trypanosome enzyme structure by molecular replacement.

The three-dimensional structure of the enzyme is required to investigate structural differences with the sheep enzyme structure and aid the design of mutants to probe the active site. It also enables the assessment of the enzyme as a target for the rational design of compounds against a variety of tropical diseases caused by trypanosomal parasites. The current state of our structural studies will be reported.

PS-03.05.24 STRUCTURAL STUDIES ON ALCOHOL DEHYDROGENASES AND INHIBITOR COMPLEXES. by Ramaswamy. S^{*1}, Bryce. V. Plapp² and Hans Eklund¹. ¹Department of Molecular Biology, Swedish University of Agricultural Sciences, Uppsala Sweden and ²Department of Biochemistry, Iowa University, Iowa USA.

As a part of the ongoing project on Alcohol Dehydrogenases, the structure of the horse liver enzyme-NAD⁺-alcohol complex, which should resemble the active Michaelis complex, was determined with X-ray crystallographic data at a resolution of 2.4 Å and refined to an R value of 18.8%. The structure is very similar to those determined previously at 2.9 Å for the triclinic, ternary complexes of the enzyme, in particular, the complex with coenzyme and p-bromobenzyl alcohol (Eklund, H., Plapp, B. V., Samama, J.-P., and Brändén, C.-I. *J. Biol. Chem.* (1982), 257, 14349-14358). The position of the 2,3,4,5,6-pentafluorobenzyl alcohol is very well-defined in electron density; the oxygen of its hydroxyl group is ligated to the catalytic zinc, which has a distorted tetrahedral configuration. The hydroxyl group is part of the hydrogen-bonded system consisting of the hydroxyl group of Ser-48, linked through the hydroxyl group of the 2' carbon of the nicotinamide ribose to the imidazole group of His-51, which can act as a base and shuttle a proton to solvent. Carbon 1 of the alcohol is about 3.8 Å from C4 of the nicotinamide ring, and positioned so that the pro-R hydrogen would be transferred after small movements of the alcohol. The tight steric interactions around the alcohol and the NAD would prevent close approach of the reacting substrates unless protein dynamics overcame the energetic barriers.

Crystallographic data has also been collected on crystals grown in the presence of Norborneol and heptafluoro butanol. The analysis of these data are in progress. Simultaneously, attempts at structure determination of the yeast enzyme, which crystallizes in the hexagonal space group of P622 is in progress. The results of these studies will be presented.

PS-03.05.25 PRELIMINARY X-RAY DIFFRACTION STUDY OF γ -GLUTAMYLTRANSPEPTIDASE FROM *E. COLI* K-12.

By Sakai, H.^{1*}, Sakabe, K.², Sakabe, N.^{1,4}, Nohara, S.³, Suzuki, H.³ and Kumagai, H.³ 1) Dept. of Synchrotron Radiation Science, Grad. Univ. Advanced Studies, 1-1 Oho, Tsukuba, Ibaraki 305, Japan. 2) Dept. of Chemistry, Faculty of Science, Nagoya Univ., Chikusa, Nagoya 464, Japan. 3) Dept. of Food Science & Technology, Faculty of Agriculture, Kyoto Univ., Sakyo-ku, Kyoto 606, Japan. 4) Photon Factory, KEK, 1-1 Oho, Tsukuba, Ibaraki 305, Japan.

γ -Glutamyltranspeptidase (GGT) (E.C. 2.3.2.2) is the enzyme which catalyzes the hydrolysis of glutathione or other γ -glutamyl compounds, and transfer the γ -glutamyl moiety to amino acids and peptides. GGT from *Escherichia coli* K-12 was purified and its biochemical properties have been studied (Suzuki, H. *et al.*, *J. Bacteriol.*, 1986, **168**, 1325-1331). It consists of a large and a small subunit with molecular weights of 39,200 (365 amino acids) and 20,000 (190 amino acids), respectively (Suzuki, H. *et al.*, *J. Bacteriol.*, 1989, **171**, 5169-5172). The aim of present investigation is to determine the three dimensional structure of GGT to gain an insight into the reaction mechanism, and also to provide a basis of relationship with other GGTs.

Crystals were grown by the vapour diffusion technique from NaAc-HCl buffer at pH 5.2 with 50mM NaCl using polyethylene glycol 6000 as a precipitant. The maximum size of the crystal is 1.0mm x 0.5mm x 0.25mm. The space group is $P2_12_12_1$ with unit cell dimensions of a=128, b=130 and c=79Å. Assuming that two GGT molecules are contained in an asymmetric unit, the V_m value is 2.8 Å³/dalton which is in the range expected from protein crystals.

Diffraction intensities were collected up to 2.5 Å resolution using synchrotron radiation and the Weissenberg camera for macromolecular crystallography equipped with an imaging plate at station BL6A2, Photon Factory, KEK (Sakabe, N. *Nucl. Instrum. Methods*, 1991, **A303**, 448-463). In order to solve the crystal structure by the multiple isomorphous replacement method, data collection of possible heavy atom derivatives have been carried out in addition to the native data. Due to the relative stability of the crystal against synchrotron X-ray radiation, a data set (more than 90° rotation around an axis) can be obtained from a single crystal. Unfortunately, the short c-axis has a tendency to shrink by up to 2Å during soaking in the heavy atom solutions tried so far. Difference Patterson and anomalous difference Patterson maps were carefully inspected, yielding a Pb-derivative. A search for further, more isomorphous, heavy atom derivatives is now in progress.

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CRYSTALLOGRAPHIC STUDIES OF ORNITHINE TRANSCARBAMOYLASE. Lei Jin*, James F. Head, Lawrence C. Kuo and Barbara A. Seaton, Department of Physiology, Boston University, School of Medicine, U.S.A.