on a rotating anode (CuKα) generator with a merging R of 9.9% (for 98793 measurements of 19667 reflections with I> 4σ). 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 enyzme 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. Plapp[2] and Hans Eklund[1]. [1]Department of Molecular Biology, Swedish University of Agricultural Sciences , Uppsala Sweden and [2]Department 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 heptafluro 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 Å^3/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.

PS–03.05.26

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.

Ornithine transcarbamoylase (OTCase) is a critical enzyme in the urea cycle. OTCase deficiency, inherited as an X-linked trait, is the most prevalent genetic defect of ureagenesis, causing severe neonatal hyperammonemia. Point mutations of the OTCase gene account for about 80-85% of the patients.

We have purified both recombinant E. coli and human liver OTCase following the procedure described previously. Single crystals of E. coli OTCase suitable for X-ray analysis (0.5-0.8 mm long) have been grown. The crystallization of the human liver OTCase is in progress.

The space group of E. coli OTCase has been determined as either $P3_1$ or $P3_2$. There is one homotrimer of 36.8 kDa subunits in the asymmetric unit. A complete native data set to 2.8 Å has been collected. The flash freezing technique using liquid nitrogen was employed during the data collection to prevent radiation damage. The unit cell dimensions are a=b=103.43 Å and c=86.45 Å. Data have also been collected on several potential derivatives to allow a multiple isomorphous replacement solution of the structure.

The result of the self-rotation function calculation indicates that there is three-fold non-crystallographic symmetry in the crystals of E. coli OTCase. At a later stage, we can use this information to average electron density and therefore improve map quality.

PS–03.05.27 RICIN A REFINED AT 1.7A

S.A. Weston, A.D. Tucker, D.R. Thatcher and R.A. Pauptit*
Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield SK10 4TG, Cheshire, England.

Ricin is an exceptionally toxic heterodimeric protein from the seeds of the castor plant, *Ricinus communis*. The B-chain is a 262-residue lectin which binds eukaryotic cell surfaces. The A-chain is a 267-residue glycosidase which enters the cytoplasm by an unknown mechanism and attacks ribosomes: it removes a specific adenine base from rRNA, thereby inhibiting protein synthesis and killing the cell. The ricin A-chain is being developed as an anticancer immunotoxin.

We have grown ricin A crystals of a new tetragonal crystal form with favourable diffraction characteristics ($P4_12_12$, $a=b=68.8$ A, $c=141.2$ A). We solved the structure by molecular replacement methods using a 2.5 A model of the ricin dimer (Katzin *et al, PROTEINS* 10, 251, 1991) available in the Protein Data Bank. Data extending to 1.7A resolution were collected at the Daresbury synchrotron on a FAST area detector. We are refining the model using simmulated annealing and least-squares methods, and we will present the refined high resolution structure.

PS–03.05.28 X-RAY STRUCTURE ANALYSIS OF ABRIN-A - A RIBOSOME-INACTIVATING PROTEIN FROM SEEDS OF *ABRUS PRECATORIUS*.

Yen-Chywan Liaw*,[1], T. Tahirov[2], S.-C. Chu[3], T.-H. Lu[2], J. Y. Lin[3], [1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan [2]Department of Physics, National Tsing-Hua University, Hsin-Tsu, Taiwan [3]Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan

Abrin-a from seeds of *Abrus Precatocius* is a ribosome inactivating protein which catalyze endohydrolysis of the N-glycosidic bond at one adenosine on the 28S rRNA (EC 3.2.2.22) consisting of A and B chains of 250 and 247 amino acid residues, respectively. The three-dimensional structure of abrin-a has been determined by X-ray crystallography to a resolution 2.8Å. Crystals of this abrin-a grow in monoclinic space group $P2_1$ at room temperature with cell dimensions a=85.1Å, b=73.1Å, c=48.2Å, and $\beta=96.6^0$. The intensity data were collected on San Diego area detector system. Initial phases were determined by the method of molecular replacement using a starting model built from QUANTA protein package with ricin structure as a template. The molecule containing 4842 atoms in the asymmetric unit has been refined by using X-PLOR to a crystallographic $R = 0.218$ for 21128 data with $I \geq 1.5\sigma(I)$. The analysis of the detailed structure is in progress.

PS–03.05.29 FLUORIDE INHIBITION OF ENOLASE: CRYSTALLOGRAPHIC STUDIES AND ELECTROSTATIC POTENTIAL CALCULATIONS. By L. Lebioda*, K. Lewinski, E. Zhang, Department of Chemistry and Biochemistry, University of South Carolina and J. M. Brewer, Department of Biochemistry, University of Georgia, U.S.A.

Enolase in the presence of its physiological cofactor Mg^{2+} is inhibited by fluoride and phosphate ions in a strongly cooperative manner (Nowak, T. & Maurer, P. *Biochemistry* 20, 6901, **1981**). The structure of the quaternary complex yeast enolase - Mg^{2+}-F^--Pi has been determined by x-ray diffraction and refined to an R=16.9% for those data with $F/\sigma(F) \geq 3$ to 2.6Å resolution with a good geometry of the model. The movable loops of Pro35-Ala45, Val153-Phe169 and Asp255-Asn266 are in the closed conformation found previously in the precatalytic substrate-enzyme complex. Calculations of molecular electrostatic potential show that this conformation stabilizes binding of negatively charged ligands at the Mg^{2+} ion more strongly than the open conformation observed in the native enolase. This closed conformation is complementary to the transition state, which also has a negatively charged ion, hydroxide, at Mg^{2+}. The synergism of inhibition by F^- and Pi most probably is due to the requirement of Pi for the closed conformation. It is possible that other Mg^{2+} dependent enzymes that have OH^- ions bound to the metal ion in the transition state also will be inhibited by fluoride ions.

PS–03.05.30 Crystallographic studies on substrate complexes of ω-amino acid:pyruvate aminotransferase by Ikemizu, S.[1,*], Rehse, P.[2], Watanabe, N.[2], Sakabe, K.[3], Sakabe, N.[1,2] and Yonaha, K.[4]

1) Department of Synchrotron Radiation Science, The Graduate University for Advanced Studies, Tsukuba, Ibaraki 305, Japan; 2)