A self rotation function calculation (Merlot program package) clearly revealed a non-crystallographic 2-fold axis which indicates the possibility of a dimeric association of the protein within the crystal. Multiple isomorphous replacement is being used to solve the structure. Three heavy atom derivatives were obtained (Eu nitrate,Gd nitrate, EtHgCl) and X-ray intensities were collected to 3.0 Å resolution on an Imaging plate system. Phasing is currently underway.

Small angle neutron scattering measurements were also undertaken in order to characterize the association state of the H protein in different solutions and also of H with the other proteins of the complex. The results will be discussed and related to biochemical studies of this complex (Oliver, Neuburger & Douce, 1990).

References

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PS–03.05.21 CRYSTALLOGRAPHIC STUDIES TOWARDS THE CATALYTIC MECHANISM OF GLYCOGEN PHOSPHORYLASE.

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Considerable time and effort has been devoted to determining the precise role of phosphate in the catalytic mechanism of glycogen phosphorylase (GP).

Previous time resolved crystallographic experiments with monochromatic radiation have allowed the reaction of heptenitol and phosphate to be followed in the crystal. One intermediate shot in this series of experiments indicated a possible attacking position of phosphate.

Binding studies in T state crystals with inorganic phosphate showed no binding at the catalytic site even at concentrations of 1M.

As part of a series of glycosidase inhibitors the nojirimycin tetrazole has been synthesised (Ph. Ermert & A.T. Vasella, 1991) and found to be a poor inhibitor of GP by itself (K_i=700μM), but in the presence of phosphate becomes one of the tightest known (K_i=50μM)).

A crystallographic binding study of the tetrazole/phosphate complex with T state GP showed phosphate to bind close to the postulated attacking position. These results have been confirmed with R state crystals which do exhibit a high affinity for dianions.

The rationale and implications for the catalytic mechanism will be discussed in the poster presentation.

PH. ERMERT & A.T. VASELLA (1991). *Helv. Chim. Acta* **74**, 2043-2053.

PS–03.05.22 TOWARDS THE STRUCTURE OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY SINGLE ISOMORPHOUS REPLACEMENT

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Leuconostoc mesenteroides glucose 6-phosphate dehydrogenase (G6PD) can utilise either NAD^+ or $NADP^+$ in the oxidation of glucose 6-phosphate, depending on the physiological conditions. This dual coenzyme specificity is unusual among dehydrogenases, most of which strongly prefer one over the other. In an attempt to understand the mechanism whereby the enzyme selects its coenzyme, and the protein structural basis for this, we are currently engaged in determining the crystal structure of the enzyme.

The enzyme is a dimer composed of two identical subunits of 485 amino acids and molecular weight ~ 54000. Crystals of the wild-type enzyme had been obtained and diffraction studies initiated, but the resulting crystals were very sensitive to radiation damage and only diffracted weakly. Furthermore, all attempts to prepare suitable heavy atom derivatives for multiple isomorphous replacement structure determination methods were unsuccessful, partly because of the absence of cysteine residues. The recent successful cloning and sequencing of the gene encoding *L.mesenteroides* G6PD, and the construction of a suitable strain of *E.coli* IH which this gene is expressed (Lee,W.T., Flynn,T.G., Lyons,C & Levy,H.R. (1991). *J. Biol. Chem.* **266**, 13028-13034.), prompted us to use site-directed mutagenesis to address this problem.

Six mutants of *L.mesenteroides* G6PD have been synthesised and characterised. In each of these either a serine or a glutamine residue has been changed to a cysteine, giving the potential for high occupancy, single site heavy atom binding.

Trigonal crystals for five of the six mutants have been grown ($P3_121$ or $P3_221$; a=b=105.7Å, c=224.3Å, α=β=90°, γ=120°), which are all isomorphous with the wild-type recombinant enzyme. These crystals are less sensitive to radiation damage, and diffract more strongly than those grown from the conventionally extracted enzyme. Co-crystallisation of the S215C mutant with sodium p-(hydroxymercuri)benzoate has yielded crystals isomorphous with the native wild-type. Crystals of this derivative have been used to collect X-ray diffraction data to 3.5Å resolution and subsequent difference Patterson analyses show that there are two mercury sites in the asymmetric unit, one in each monomer. To date, none of the other mutants have given any promise of heavy metal binding.

Refinement of the mercury sites, phasing, and then solvent flattening allowed the calculation of a 3.5Å resolution electron density map of sufficient quality to observe that our original choice for the enantiomorphic space group, $P3_121$, was wrong, as the map contained left-handed helices. Fitting some of the most easily interpretable areas of the $P3_221$ map allowed us to determine the non-crystallographic two-fold dimer axis. Using this axis, averaging techniques have further improved the map and it has now proved possible to trace a large portion of the protein density. Phase combination techniques are currently in progress and it is hoped to collect anomalous dispersion data to improve the phases further.

PS–03.05.23 CRYSTALLOGRAPHIC STUDIES ON 6-PHOSPHOGLUCONATE DEHYDROGENASE FROM *TRYPANOSOMA BRUCEI*

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Sleeping sickness in man and a number of economically important veterinary diseases in sub-Saharan Africa are caused by sub-species of the parasitic protozoan *Typanosoma brucei* (*T.brucei*). The treatment of these conditions is unsatisfactory and less toxic, more effective drugs are being sought. The accelerated rate of glucose metabolism exhibited by the trypanosome while in the host's bloodstream suggests that its metabolic enzymes may prove viable targets for drug design.

We have recently crystallized recombinant *T.brucei* 6-phosphogluconate dehydrogenase (6-PGDH), the third enzyme of the oxidative branch of the pentose phosphate pathway. The crystals are trigonal, space group $P3_121$ or $P3_221$, with unit cell parameters a=b=135.1Å, c=116.7Å, α=β=90°, γ=120° and are stable in an X-ray beam . We have collected a 96% complete data set to 2.8Å resolution

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.

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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.