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-nitrato,Gd nitrate, EuF3Cl) 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


PS-03.55.21 CRYSTALLOGRAPHIC STUDIES TOWARDS THE CATALYTIC MECHANISM OF GLYCOPEN PHOSPHORYLASE, E.P. Mitchell*, L.K. Johnson, Laboratory of Molecular Biophysics, Rex Richards Building, University of Oxford, South Parks Road, Oxford OX1 3QU, England.

One intermediate shot in his series of experiments indicated a possible stacking pattern of phosphate. Binding studies in T state crystals with inorganic phosphate showed no binding at the catalytic site even when concentrations of 1M. As part of a series of glycogen inhibitors the Najjarin effect has been synthesized (Ph. Ermer & A.T. Vasella, 1991) and found to be a poor inhibitor of GP by itself (K_i = 2000M), but in the presence of phosphate becomes one of the tightest known (K_i = 0.5M).

A crystallographic binding study of the ternary/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 dinucleotides. The rationale and implications for the catalytic mechanism will be discussed in the poster presentation.


PS-03.55.22 TOWARDS THE STRUCTURE OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY SINGLE ISOMORPHOUS REPLACEMENT

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Leucosedos mesneroides 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 favor one over the other. In an attempt to understand the mechanism whereby the enzyme selects its coenzyme, and the proton 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 466 amino acids and molecular weight 54600. Details of the wild-type enzyme had been obtained and diffraction studies initiated, but the resulting crystals were very sensitive to radiation damage and only diffused 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. mesnerioides G6PD, and the construction of a suitable strain of E.coli it which this gene is expressed (Lee, W.T.; Flynn, T.G., Lignos & 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. mesnerioides G6PD have been synthesised and characterised. In each of the four either a section of a glucose or of glutamine 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_212, a = 105.7Å, c = 224 Å, β = 90°, γ = 120°), which are all isomorphous with the wild-type recombinant enzyme. These crystals are less sensitive to radiation damage, and diffuse more strongly than those grown from the conventionally extracted enzyme. Co-crystallisation of the P3_212 with sodium hydroxymercuribenzoate 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 has given any precession data, and 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 all but one original choice for the mienoamorphic space group P3_212, was wrong, and the map contained left-handed helices. Pitting some of the most easily interpretable areas of the P3_212 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-03.55.23 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 parasite Trypanosoma brucei (Tr. brucei). The treatment of these conditions is unsatisfactory and lacks 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 Tr. brucei 6-phosphogluconate dehydrogenase (6PGDH), the third enzyme of the oxidative branch of the pentose phosphate pathway. The crystals are trigonal, space group P3_121 or P3_212, with unit cell parameters a = 135.5Å, b = 135.5Å, c = 135.5Å, β = 90°, γ = 120° and are stable in an X-ray beam. We have collected a 95% complete data set to 2.8Å resolution.