03-Crystallography of Biological Macromolecules

PS–03.05.08 STRUCTURAL STUDIES OF GLUCOSE DEHYDROGENASE FROM A THERMOPHILIC ARCHAEON. J.J. John*, G.L. Taylor, M.J. Danson, D.W. Hough; Department of Biochemistry, University of Bath, Bath, BA2 7AY, U.K.

Glucose dehydrogenase, a dual cofactor specific enzyme (NADP/NAD) from *Thermoplasma acidophilum* catalyses the first reaction in a non-phosphorylated Entner-Doudoroff pathway. It shares low sequence homology with other bacterial glucose dehydrogenases (<20%) , although a tentative link with "long chain zinc containing alcohol dehydrogenases" has been observed. Although crystallisation of glucose dehydrogenase was previously reported (Bright et al, 1991), these crystals were found to be disordered. Subsequent improved purification and extensive crystallisation trials, including the Incomplete Factorial approach, yielded small but regular crystals using polyethylene glycol 4000/ propanol. The repeated macroseeding technique led to an increase in size of the crystals, which were suitable for X-ray analysis. The protein crystallises in the monoclinic spacegroup $P2_1$, with cell dimensions a = 80Å b = 119.5Å c = 88Å and with a tetramer in the asymmetric unit. Data were collected on a Siemens rotating anode and at station 9.5 of the Daresbury synchrotron source.
If molecular replacement with horse liver alcohol dehydrogenase proves unsuccessful, structure determination using the isomorphous replacement method will be employed; to this end, a search for heavy atom derivatives is in progress.

Bright, J. , Mackness, R. , Danson, M. , Hough, D. , Taylor, G. , Towner, P. , Byrom, D. (1991). J. Mol. Biol 222, 143-144

PS–03.05.09 THE STRUCTURE OF SALMONELLA TYPHIMURIUM LT2 NEURAMINIDASE AT 1.6Å. S.J. Crennell*, G.L. Taylor, E.F. Garman§, W.G. Laver† and E. Vimr‡, Department of Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.

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The structure of *S. Typhimurium* LT2 Neuraminidase has been determined using heavy atom isomorphous replacement. Neuraminidases cleave the terminal sialic acid from glycoconjugates. The freed sialic acid is sometimes used as a carbon source by the bacterium. Previous work has focussed heavily on viral neuraminidases, the structures of various influenza A and B subtypes having been reported. The sequence homology between viral and bacterial neuraminidases is only 16% and an attempt to determine the *S. Typhimurium* structure by molecular replacement using the viral structure was not successful.
Derivatives were prepared using pCMB and $HgCl_2$ solutions, refining of Hg positions and phasing took place in MLPHARE. The phases were improved with B.C.Wang's solvent flattening method despite the low solvent content (42%). The graphics program "O" was used for interpretation of the MIR map and the sequence aligned using the Hg sites as CYS markers. The structure was refined using "XPLOR" and water positions found in 2Fo-Fc maps.
The structure will be presented and compared with that of viral neuramindases.

PS–03.05.10 STRUCTURE OF TRYPANOTHIONE REDUCTASE FROM TRYPANOSOMA CRUZI. By Y. Zhang[1],S. Bailey[1], A.H.Fairlamb[2] and W. N. Hunter[1]
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Chagas' disease caused by Trypanosoma cruzi infects millions of people in central and south America. Current treatment for this life threatening disease is unsatisfactory, new and more effective drugs are urgently needed. Trypanothione reductase (TR) is an enzyme unique and essential to trypanosomes where it catalyzes the reduced b - nicotinamide adenine dinucleotide phosphate (NADPH) _ linked reduction of trypanothione, N^1 ,N^8 - bis (glutathionyl) spermidine, $T(S)_2$, a glutathione analog unique to trypanosomatid parasites (Alan H. Failamb, Annu. Rev. Microbiol, 1992,46,695-729):

$$NADPH+H^+ + T(S)_2 = NADP^+ + T(SH)_2$$

TR represents a prime target for the rational grug design of new compounds suitable for the treatment of trypanosomaisis.
Trypanthione Reductase from Trypanosoma cruzi has been cloned, overproduced and purified. Well ordered crystals of TR were obtained by hanging drop vapor diffusion techniques. Diffraction data to 2.7 Å were recorded at the Daresbury Synchrotron on station PX9.5. Space group $P4_3$, unit cell dimension a=b=92.8Å, c=156.6Å, with one dimer per asymmetric unit.
The crystal structure of TR was solved by molecular replacement method(MERLOT) using a truncated model of C.fasciculata TR. Rotation function and translation function calculation yield interpretable results. Structure refinement is in process.Details of the refinement and the structure will be presented.

PS–03.05.11 THE REFINED STRUCTURE OF PYRUVATE KINASE AT 2.6Å. S.C. Allen* and H. Muirhead.§

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Pyruvate kinase catalyses the second ATP generating reaction of glycolysis. A phosphate group is transferred from phosphoenolpyruvate to Mg.ATP, producing pyruvate, Mg.ATP and a proton. There are several pyruvate kinase isoenzymes, some of which are allosterically regulated, so this reaction is a point at which flux through the glycolytic pathway may be controlled. The molecular basis for allosteric regulation has been successfully investigated with a number of enzymes, where the high substrate affinity (R) state and low substrate affinity (T) state structures have been elucidated by X-ray crystallographic techniques.
Here, we report the refined structure of an R state pyruvate kinase; the M1 isoenzyme from cat muscle, with the intention of using this as a basis for understanding the allosteric mechanism. Work to crystallize a T state enzyme is continuing. The structure of the non-allosterically regulated M1 isoenzyme has been refined using "X-PLOR" to a resolution of 2.6Å, and the final crystallographic R factor for all data is 20.4%, with good geometrical parameters. The inter-subunit interactions have been analysed, and compared with models of allosterically regulated isoenzymes, constructed using the M1 structure as a starting point.

