PS-03.05.09 THE STRUCTURE OF SALMONELLA TYPHIMURIUM LT2 NEURAMINIDASE AT 1.6 Å. S.C. Crennell*, G.L. Taylor, E.F. Garman, W.G. Laver, and E. Vinet, Department of Biochemistry, University of Bath, Claverdon 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 terminal sialic acid is sometimes used as a carbon source by the bacterium. Previous work has focused 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 viral templates was unsuccessful. Derivatives were prepared using cCMS and HgO3 solutions, refining of Hg positions and phasing took place in MAPHARE. 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 CVS 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 neuraminidases.

PS-03.05.10 THE REFINED STRUCTURE OF PYRUVATE KINASE FROM M. THERMOPHILUS AT 2.6 Å. S.C. Allen* and H. Mairhead,§
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Pyruvate kinase catalyzes the second ATP generating reaction of glycolysis. A phosphate group is transferred from phosphoenolpyruvate to MgATP, producing pyruvate, MgATP 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 one 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 "XPLOR" to a resolution of 2.6 Å, and the final crystallographic R factor for all data is 20.4%, with good geometrical parameters. The inter-subunit interactions have been analyzed, and compared with models of allosterically regulated isoenzymes, constructed using the M1 structure as a starting point.