03-Crystallography of Biological Macromolecules

03.05.09 THE STRUCTURE OF SALMONELLA TYPHIMURIUM LT2 NEURAMINIDASE AT 1.6 Å. S.J. Greensell*, G.L. Taylor, E.F. Gurney, W.G. Laver and E. Vinit, Laboratory of Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.

The structure of S. Typhimurium LT2 Neuraminidase has been determined using heavy atom isomorphous replacement. Neuraminidases cleave the terminal sialic acid from glycoconjugates. The initial crystallization conditions proved unsuccessful, so structure determination using the isomorphous replacement method was employed. 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.

03.05.10 THE RENEFINED STRUCTURE OF PYRUVATE KINASE AT 2.6 Å. S.C. Allen* and H. Mairhead. Department of Biochemistry, University of Bristol, Bristol, BS8 2TD, U.K.

Pyruvate kinase catalyzes 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 P. pyruvate kinase isoenzymes, one of which is allosterically regulated so that the reaction is at 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 (E) 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 isozyme 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 isozyme 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.