PS04.01.48 THE X-RAY STRUCTURE OF URIDINE PHOSPHORYLASE FROM E. coli AT 2.5 Å RESOLUTION. A. M. Mikhailov, E. Yu. Morgunova, Institute of Crystallography, Moscow, Russia; S. E. Ballek, Cs. Maes, St. R. Armstrong, Cornell University, NY 14853, USA; A. S. Mironov, A. A. Komissarov, St. Petersburg, Russia

Uridine phosphorylase (EC 2.4.2.3; UPhase) from E. coli catalyzes the reversible phosphorylisis of uridine with the formation of ribose-1-phosphate and uracil. UPhase has been identified as the enzyme which is responsible for the cleavage of some pyrimidine nucleoside analogs towards antitumor activity. The enzyme molecule is homotetramer with point symmetry 32. The molecular weight is 165 KDa. The primary structure of the subunits is known and includes 253 aminoacid residues. UPhase structure was solved at 2.5 Å resolution by molecular replacement method. The crystallographic refinement was performed by the program X-PLOR to R-factor 18.6%. Rms deviations from bond length of 0.012 Å and bond angles 2.095°. The 150 water molecules were picked in the structure.

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PS04.01.49 A GLIMPSE AT AN ENZYME REACTION INTE­MEDIATE: THE ATP-Mg2+-OXALATE TERNARY COMPLEX OF ESCHERICHIA COLI PHOSPHO­NOLPYRUVATE CARBOXYKINASE. Leslie W. Tari, Allan Matte, Umarani Pugazhenthi, Hughes Goldie and Louis T. J. Delbaere, Departments of Biochemistry and Microbiology, University of Saskatchewan, Saskatoon Saskatchewan, Canada S7N 0W0

Phosphonolpyruvate carboxykinase (PCK; EC 4.1.1.49) is a key metabolic enzyme which catalyzes the reaction representing the first committed step in the diversion of tricarboxylic acid cycle intermediates towards gluconeogenesis, the conversion of oxaloacetate to phosphoenolpyruvate. By molecular replacement, the crystal structure of adenosine triphosphate (ATP)-magnesium-oxalate bound PCK from Escherichia coli (M, 59533, 540 residues) has been solved, using the structure of native PCK as a search model. Refinement by energy-restrained least-squares, simulated annealing, manual model building and map re-fitting converged at an R-factor of 19.5% using 35505 6-0.1 Å data (76% complete). ATP binding induces a large hinge-like rotation of the N- and C-terminal domains which closes the active site cleft. PCK possesses a novel nucleotide binding fold, particularly in the adenine-binding region, where the formation of a cis backbone torsion angle in a loop glycine promotes intimate contacts between the adenine-binding loop and adenine, while stabilizing a syn-conformation of the purine base. This complex represents a reaction intermediate analog along the pathway of the conversion of oxaloacetate to phosphoenolpyruvate, and provides insight into the mechanistic details of the chemical reaction catalyzed by this enzyme.

PS04.01.50 CRYSTAL STRUCTURE OF ENZYME IIA OF THE MYCOPLASMA PHOSPHOTRANSFERASE SYSTEM. Kui Huang1, Geeta Kapadia1, Peng-Peng Zhu2, Alan Petercow2 and Osmar Herzberg1, 1Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland, 20850; 2Laboratory of Biochemical Genetics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, 20892.

The 2.5 Å resolution crystal structure of the IIA domain of the glucose permease from Mycoplasma capricolum has been determined by the molecular replacement methods, using the structure of the Bacillus subtilis IIA domain as the search model (40% sequence identity). This 154 residue protein is one of the three domains comprising the entire Mycoplasma enzyme II, which accepts a phosphoryl group from HPr and transfer it to a sugar, via two phosphorylation/dephosphorylation steps. Whereas the overall structure of the Mycoplasma enzyme IIA is similar to those from B. Subtilis and E. coli, the N-terminal and the C-terminal fragments adopt a conformation different from either. The phosphorylation site, His78, is coordinated to a Zn2+ ion which was present in the crystalization solution. Interestingly, the Mycoplasma glucose IIA domain accepts and transfers a phosphoryl group from the phosphotransferase systems of B. Subtilis and E. coli. The crystal structure of the Mycoplasma IIA will be compared in detail with those from B. Subtilis and E. coli, and a model of the complex between enzyme IIA and HPr will be presented, in an effort to understand the mechanism underlying the cross reactivity between Mycoplasma and the bacterial phosphotransferase systems.

PS04.01.51 1.5Å STRUCTURE OF THE ASP46 HPr MUNT FROM ESCHERICHIA COLI. Scott Napper, Bruce Waygood, J. Wilson Quail, Louis T. J. Delbaere, Departments of Biochemistry and Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan.

Regulation of the phosphoenolpyruvate:sugar transferase system (PTS) in bacteria differs in Gram-positive to Gram-negative species. Gram-positive bacteria possess a regulatory mechanism at the level of HPr which is not seen in Gram-negative bacteria. Gram-positive sugar transport function can be inhibited through reversible phosphorylation of a conserved Ser46 residue of HPr. The kinase catalyzing this reaction is absent from Gram-negative bacteria and E. coli HPr is unable to be phosphorylated by it in vitro. The Asp46 mutant was created in an attempt to mimic this regulatory phosphorylation event through similar introduction of negative change. The mutant shows very similar properties to the phosphorylated Ser46 HPr in diminishing phosphorylation activity. The crystallographic structure of this mutant has been determined through the method of molecular replacement and refined to a conventional R-index of 18.9%. The crystal structure shows that this inhibition occurs in the absence of any structural alterations. Rather it appears as though changes in the electrostatic surface potential are responsible for the inability of the protein to interact with other proteins, in particular Enzyme I.