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. Ealick, Ch. Mao, Sh. R. Armstrong, Cornell University, NY 14853, USA; A. S. Mironov, A. A. Komissarov, Gniigenetica, Moscow, Russia

Uridine phosphorylase (EC 2.4.2.3; UPhase) from E.coli catalyzes the reversible phosphorolysis of uridine with the formation of riboso-1-phosphate and uracil. UPhase has been identified as the enzyme which is responsible for the cleavage of some pyrimidine nucleoside analogs possessing the antitumor activity. The enzyme molecule is hexamer 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. The UPhase monomer is an α/β protein. Six a-helixes and two twisted β-sheets form the main core of the monomer. There are two flexible loops which play the important role in the enzyme catalyze. So, the flexible loop (residues Tyr163-Phe180) extends into the neighboring subunit. This loop is hydrophobic and five hydrogen bonds were found between this loop and neighboring subunit. The active site is located near the interface of two subunits. This region includes residues 26-30, 68-70, 91-96, 162-163, 166-168, 195-198 and 220-223 of one subunit and residues 5-8 of another. Recent studies by selective chemical modification have indicated that Asp-5, Cys-136 and Tyr-169 are present near or in the UPhase active site.

PS04.01.49 A GLIMPSE AT AN ENZYME REACTION INTERMEDIATE: THE ATP-Mg^{2+}-OXALATE TERNARY COMPLEX OF *ESCHERICHIA COLI* PHOSPHOENOLPYRUVATE 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

Phosphoenolpyruvate carboxykinase (PCK; E. C. 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_r 59583, 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 35508 6.0-1.8 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 Huang[1], Geeta Kapadia[1], Peng-Peng Zhu[2], Alan Peterkofsky[2] and Osnat Herzberg[1], [1]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, Maryland, 20850; [2]Laboratory of Biochemical Genetics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, 20892.

The 2.5 Å resoluiton 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 Zn^{2+} ion which was present in the crystallization 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 MUTANT 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 attempt to mimic this regulatory phosphorylation event through similar introduction of negative charge. The mutant shows very similar properties to the phosphorylated Ser46 HPr in diminishing phosphotransfer 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.