Acta Cryst. (2002). A**58** (Supplement), C95

CRYSTAL STRUCTURE OF L-HOMOCYSTEINE γ-LYASE AT 1.8 Å

T. W. Allen[1] V. Prasad[2] G. S. Prasad[2] R. M. Hoffman[2] S. Ramaswamy[1]
[1]University of Iowa Department of Biochemistry Bowen Science Building 51 Newton Road IOWA CITY IOWA 52242 USA [2]AntiCancer, Inc., San Diego, California, USA

Numerous malignant cell lines, including those responsible for some gastric and colon cancers, have an elevated dependence on methionine relative to ordinary cells. Treatment of these cancers with enzymes that reduce free methionine has been shown to slow or arrest tumor growth. When deprived of methionine, cells can synthesize it from the similar amino acid homocysteine. We have recently solved the structure of the enzyme L-homocysteine α, γ-hydrolyase (obtained from *Trichomonas vaginalis*) which transaminates homocysteine. The structure of homocysteinase is similar to that of other α, γ-hydrolases such as L-methionine α, γ-hydrolyase. In conjunction with methioninase, homocysteinase is expected to enhance cancer treatment, especially if its activity and half-life can be increased by mutation. Further investigation of the interactions involved, such as the binding of pyridoxal 5' phosphate (PLP), will elucidate the mechanisms of this type of enzyme.

Keywords: HOMOCYSTEINE METHIONINE CANCER

Acta Cryst. (2002). A**58** (Supplement), C95

CRYSTAL STRUCTURE OF FULL LENGTH HPr KINASE/PHOSPHATASE FROM *MYCOPLASMA PNEUMONIAE*

G. Allen[1] K Steinhauer[2] W HIllen[2] J Stuelke[2] RG Brennan[1]
[1]Oregon Health & Sciences University Biochemistry & Molecular Bioloby 3181 SW Sam Jackson Park Rd. Mrb534 PORTLAND OREGON 97201 USA [2]Lehrstuhl fur Mikrobiologie, Friedrich-Alexander Universitat, Erlangen-Nurnberg

Presumably to maximize their energy efficiency, bacteria coordinate the expression of metabolic enzymes with the types of carbohydrates available in the surrounding environment. This mechanism, called Carbon Catabolite Repression (CCR), has been extensively studied in *E. coli* and other gram-negative bacteria since Monod first noted that *E. coli* prefer glucose to lactose. The phenomenon of CCR also occurs in gram-positive bacteria but is affected through different mechanisms. In Bacillus subtilis, and other gram-positive bacteria, transcription of secondary metabolic enzymes (i.e. non-glucose utilizing) is repressed through the binding of the complex of Catabolite Control Protein A and phospho-Ser46-HPr (Histidine protein) to 16 base pair pseudo-palindromic sites, called Catabolite Response Elements, found in the operons regulated by glucose. In response to high levels of fructose 1,6 bis-phosphate, a glycolytic intermediate, or inorganic phosphate, HPr kinase/phosphatase (HPrK/P) phosphorylates or dephosphorylates HPr at S46.
HPrK/P from *Mycoplasma pneumoniae* forms a homo-hexamer, of which each monomer contains 2 domains connected by a short loop. The N-terminal domain, the function of which is uncertain, contains residues 1' 132 and is most closely related to UDPMurNAc-tripeptide d-alanyl-d-alanine-adding enzyme (MurF) from *E. coli*. The C-terminal domain contains the well-described P-loop (Walker A box) ATP binding motif and takes a fold similar to *E. coli* phospho-enolpyruvate carboxykinase. An analysis of the kinase and phosphatase activities of several site-directed substitutions will be discussed in the framework of the C-terminal domain.

Keywords: **KINASE PHOSPHATASE ENZYME**

Acta Cryst. (2002). A**58** (Supplement), C95

STRUCTURE DETERMINATION OF URACIL PHOSPHORIBOSYLTRANSFERASE FROM *SULFOLOBUS SOLFATARICUS*

S. Arent[1] P. Harris[1] K.F. Jensen[2] S. Larsen[1]
[1]University of Copenhagen Centre For Crystallographic Studies Department of Chemistry Universitetsparken 5 COPENHAGEN DK-2100 DENMARK
[2]Institute of Molecular Biology, University of Copenhagen, Denmark

Uracil phosphoribosyltransferase (UPRTase) catalyzes the formation of uridine monophosphate (UMP) and pyrophosphate from uracil and 5-phosphoribosyl-α-1-pyrophosphate (PRPP). UMP is the precursor for all pyrimidine nucleotides, and the UPRTase catalyzed reaction salvages pre-formed pyrimidines as a supplement to the de novo synthesis. UPRTase from *Sulfolobus solfataricus* is regulated allosterically by the nuclotides GTP and CTP, and inhibited by its product UMP. The structure determination of UPRTase in complex with UMP is in progress. Data were collected to 2.3 Å resolution on a selenomethionine substituted protein crystal. Initial phasing was obtained by the single wavelength anomalous dispersion method. Four monomers is contained in the asymmetric unit. The positions of the selenium sites indicate a homodimer of asymmetric dimers as the quarternary structure present in the crystal. Currently a model containing 2/3 of the enzyme sequence has been refined (R = 39%, Rfree = 44%). Furthermore, *S. solfataricus* UPRTase has been crystallized in complex with the allosteric activator GTP and with the allosteric inhibitor CTP.

Keywords: **ALLOSTERIC REGULATION, NUCLEOTIDE METABOLISM, PYRIMIDINE SALVAGE PATHWAYS**

Acta Cryst. (2002). A**58** (Supplement), C95

COMPARISON OF E1 FROM THE *E. COLI* PYRUVATE DEHYDROGENASE COMPLEX WITH *S. CEREVISIAE* TRANSKETOLASE

P. Arjunan[1] M. Sax[1] W. Furey[1] N. Nemeria[2] F. Jordan[2]
[1]Veterans Affairs Medical Center Biocrystallography Laboratory University Drive C Bldg 6, PO Box 12055 PITTSBURGH PA 15240 USA [2]Dept. of Chemistry, Rutgers University, 73 Warren St., Newark, NJ, 0710

Thiamin diphosphate (ThDP) is a coenzyme for a number of biochemical reactions involving cleavage of the carbon-carbon bond adjacent to a keto-group. The pyruvate dehydrogenase multienzyme complex, a member of the α-keto acid dehydrogenase family, contains multiple copies of three enzymatic components: pyruvate dehydrogenase (E1); dihydrolipoamide acetyltransferase (E2); and lipoamide dehydrogenase (E3). The three catalytic components work sequentially catalyzing the decarboxylation of pyruvate to form acetyl-CoA and carbon dioxide in an irreversible reaction. The thiamin diphosphate dependent E1 component catalyzes the decarboxylation of pyruvate to 2-α-hydroxyethylidene-ThDP and subsequent acetyl transfer to a lipoyl-lysine residue from E2. Another thiamin dependent enzyme, transketolase (TK), catalyses ketol transfer from a ketose to an aldose sugar. E1 and TK are both homodimeric enzymes with low but significant sequence homology. The E1 subunit contains 886 amino acids and the TK subunit contains 680 amino acids. The polypeptide chains of both enzymes can be divided into three domains. The structural comparison reveals that while there is general similarity, there are large differences in the N-terminal domains from both enzymes. Nevertheless, the binding mode and conformation of the cofactor ThDP are very similar. Out of twenty amino acids critical for cofactor binding and involved in cofactor activation, ten were found to be conserved. Alignment of the two enzymes indicated 541 C-α-atoms are structurally equivalent with a root mean square deviation of 2.0 Å. The comparison also enabled identification of amino acids disordered and unobserved in E1 but possibly involved in catalysis.

Keywords: **ENZYME THIAMIN DEHYDROGENASE**