

**MS-03.05.06 STRUCTURE AND FUNCTION OF GLUTATHIONE SYNTHETASE.** By H. Yamaguchi\*, H. Kato†, T. Hibi†, T. Tanaka†, T. Nishioka†, J. Oda†, and Y. Katsube, Institute for Protein Research, Osaka University, Suita, Osaka 565, Japan and †Institute for Chemical Research, Kyoto University, Uji Kyoto 611, Japan.

Glutathione synthetase (GSHase) catalyzes the synthesis of glutathione from  $\gamma$ -Glu-Cys and glycine in the presence of ATP and magnesium ion. The enzyme from *E. coli* B is a tetramer of four identical subunits of 316 amino acid residues. The reaction catalyzed by GSHase is supposed to proceed give  $\gamma$ -Glu-Cys-phosphate as an intermediate (Meister, In *The Enzymes* (Boyer, P.D., ed.), 1974, vol. 10, pp. 671 - 697, Academic Press, New York).

The crystal structure of the GSHase has been determined at 2.0 Å resolution with the crystallographic *R*-factor of 18.6 % for 17914 reflections with  $F > 3\sigma$  between 6.0 and 2.0 Å (Yamaguchi *et al.*, *J. Mol. Biol.*, 1993, 230, in press). In the tetrameric molecule, two subunits that are in close contact form a tight dimer, two tight dimers forming a tetramer with two solvent regions. The subunit structure consists of three domains: the N-terminal, the central, and the C-terminal domains. Two regions, Gly164 to Gly167 and Ile226 to Arg241, are invisible on the electron density map. The ATP molecule is located in the cleft between the central and the C-terminal domains, and its binding site is surrounded by two sets of the structural motif that belong to those respective domains. Each motif consists of an anti-parallel  $\beta$ -sheet and a glycine-rich loop. The ATP binding site has no  $\beta\alpha\beta$  nucleotide binding fold.

The peptide chain from Ile226 to Arg241 has the amino acid sequence of IPQGGETRGNLAAGGRG and is believed to form a flexible or disordered loop. This invisible loop connects neighboring  $\beta$ -strands. The direction of the two strands suggests that the loop extends over the active site and covers the bound substrates. The loop is extremely susceptible to limited proteolysis by using arginylendopeptidase and trypsin in the absence of the substrates. Only the peptide bond between the Arg233 and Gly234 was cleaved. Further, binding of substrates prevents the loop from being digested (Tanaka *et al.*, *Biochemistry*, 1992, 31, 2259 - 2265). The loop is considered to be very mobile in the absence of the substrates, but less mobile in their presence so that it closes down over the substrates bound at the active site.

In order to modify the loop flexibility, mutant enzymes were prepared by replacing a residue in the loop to another. The loop deletion mutant (DEL), in which 15 residues from Ile226 to Arg241 were replaced by three Gly residues, was also prepared. The activity of G229A, G229V, G240A and P227V mutants is 30 to 40% of that of the wild-type, while the G240V and DEL mutants are almost inactive. The structure determination of these two mutants showed that the mutant structures were identical essentially to the wild type structure. These results suggest that the flexible loop will play an essential role for the enzymatic reaction.

The enzymatic reaction proceeds smoothly when glycine attacks the intermediate,  $\gamma$ -Glu-Cys-phosphate. However, in the solvent region, there are water molecules which work as a nucleophilic reactant. If water molecules instead of glycine attack the intermediate, the enzymatic reaction no longer proceeds smoothly toward the preferable product, because the intermediate may be hydrolyzed by the water molecules. The role of the flexible loop is summarized as follows. On the binding of substrates, the flexible loop moves so as to shield the intermediate from the solvent and interacts with it. This movement of the flexible loop may prevent the acylphosphate intermediate from nonspecific attacks of water molecules.

**PS-03.05.07 X-RAY STRUCTURE OF NUCLEOSIDE DIPHOSPHATE KINASES.** By Joël Janin, Christian Dumas, Solange Moréra, Laboratoire de Biologie Structurale, Bât. 433, Université Paris-Sud, 91405-Orsay, France, and Ioan Lascu, Marie-Lise Lacombe, Michel Véron, Unité de Biochimie Cellulaire, Institut Pasteur, 75724-Paris 15, France

Nucleoside diphosphate kinases (NDP kinases) are ubiquitous enzymes that catalyze the transfer of a  $\gamma$ -phosphate from a nucleoside or deoxynucleoside triphosphate to a diphosphate. The reaction has a *ping-pong* mechanism, with a phospho-histidine intermediate. NDP kinase from *Dictyostelium discoideum* was cloned and expressed in *E. coli*. We determined the X-ray structure of a point mutant in which the active site histidine was replaced with a cysteine. Diffraction data were taken at  $\lambda=0.98$  Å on one native and one Hg derivative crystal at the LURE-DCI W32 station in Orsay. The atomic model of the H122C protein, built in the resulting high-quality SIRAS electron density map, was refined to a *R* factor of 0.20 at 2.2 Å resolution (Dumas *et al.*, 1992, EMBO J. 11:3203-3208). Data were also collected under similar conditions on crystals of wild type protein and on several complexes with nucleotides. These being non-isomorphous with the H122C protein, structure determination by molecular replacement is under way.

NDP kinase is a hexamer with D3 symmetry and a novel mononucleotide binding fold. Each 155 residue subunit contains an  $\alpha/\beta$  domain with a 4-stranded antiparallel  $\beta$ -sheet and a C-terminal extension of 17 residues. The topology is different from adenylate kinase or the p21 ras oncogen protein, and NDP kinase does not have the classical nucleotide binding loop present in these two proteins. Rather, its  $\alpha/\beta$  domain resembles the allosteric domain of regulatory subunits in *E. coli* aspartate carbamoyl-transferase (ATCase). The allosteric domain binds ATP and CTP at a position equivalent to the active site of NDP kinase, and it forms similar dimer contacts.

Trimer contacts in the NDP kinase hexamer involve a large loop of polypeptide chain. The loop bears the site of the Pro $\rightarrow$ Ser substitution in *Kpn* (*Killer of prune*) mutants of *Drosophila* NDP kinase. *Drosophila* NDP kinase is the product of the *awd* developmental gene, and it is highly homologous to the *Dictyostelium* enzyme. The *Kpn* mutant protein is fully active, but less thermostable than the wild type. Structural effects of the mutation are being explored on both the *Dictyostelium* and the *Drosophila* proteins. Progress on a 2.5 Å X-ray structure of *Drosophila* NDP kinase currently under way, should shed light on the peculiar phenotype of *Kpn*, a dominant lethal mutation in *prune* flies, suggesting that NDP kinase interacts with the *prune* gene product. Properties of human NDP kinase A and B isoforms, product of the *nm23* genes implicated in tumorigenesis, will be discussed in view of the 3-dimensional structure and of possible interactions of NDP kinase with other nucleotide binding proteins.

