P.04.02.64

Acta Cryst. (2005). A**61**, C195

Structure of γ-glutamylcysteine Synthetase Complexed with Buthionine Sulfoximine

Hiroshi Nii, Takao Hibi, Mikio Nakayama, Jun'ichi Oda, *Department of Bioscience, Fukui Prefectural University, Fukui, Japan.* E-mail: s0493001@s.fpu.ac.jp

γ-Glutamylcysteine synthetase (GCS) catalyzes the first and rate-limiting step of biosynthesis of a ubiquitous tripeptide glutathione and is a target for development of potential therapeutic agents against parasites and cancer. L-Buthionine-(*SR*)-sulfoximine (BSO) is a well-known potent inhibitor of GCS. Clinical trials of BSO have been carried out against alkylating or platinating agent resistance cancers. Crystallographic analyses of GCS-BSO complex will provide an important clue to the catalytic mechanism and structure-assisted drug design for any species of GCSs.

The crystal of *E. coli* GCS in complex with BSO belongs to the space group $P2_1$ with unit cell constants of a=70.5 Å, b=97.6 Å, c=102.7 Å and β=109.5°. The current model was refined to an R-factor of 21% (R_{free}=24%). g-Phosphate of ATP has already been transferred to the NS sulfoximine nitrogen atom of BSO. We have shown that the cysteine-binding site of the GCS is inductively formed at the binding of cysteine substrate with turn of side chains of Tyr-241 and Tyr-300 to make hydrogen bonds with the carboxyl group of cysteine that w-carboxyl group of BSO mimics. The binding of BSO to the enzyme induces the turn of the side chain of Tyr-241 in spite of the lack of BSO's w-carboxyl group. This conformational change of the side chain may be stabilized by van der Waals interaction between the side chain of Tyr-241 and the glutamate moiety in BSO.

Keywords: buthionine sulfoximine, drug design, glutathione

P.04.02.65

Acta Cryst. (2005). A**61**, C195

γ-Glutamylcysteine Synthetase: Peptide Formation Coupling with ATP Hydrolysis

Takao Hibi[a], Mikio Nakayama, Hiroshi Nii, Jun'ichi Oda, *Department of Biochemistry, Fukui Prefectural University, Fukui 910-1195, Japan.* E-mail: hibi@fpu.ac.jp

γ-Glutamylcysteine synthetase (GCS), a rate-limiting enzyme in glutathione biosynthesis, plays a central role in glutathione homeostasis and adaptive responses to stress conditions that increase the levels of reactive oxygen species. The catalytic mechanism of GCS involves the initial activation of the γ-carboxyl group of L-Glu by ATP-phosphorylation to form a γ-glutamylphosphate intermediate, followed by the nucleophilic attack of L-Cys to generate a tetrahedral transition state. In order to capture the transient steps of the catalytic mechanisms coupling between peptide formation and ATP hydrolysis, we determined the five crystal structures of GCS in complex with substrates, transition-state analogs and products.

Positional shifts of the phosphate group transferred from ATP and the bound magnesium ions induce conformational changes of two variable arms (residues 105-144 and 240-298) [1]. These arm's movements cause the side chain of Tyr-300 to turn and form a hydrogen bond with cysteine substrate, allowing the amino group of the cysteine to approach the proposed γ-glutamylphosphate intermediate. The changes of binding modes of nucleotide and amino acid substrates and the corresponding protein structural changes are correlated with the sequence of events occurring along the reaction coordinate and suggests the interesting mechanism of coupling between phosphate transfer and peptide formation.

[1] Hibi *et al*., *Proc. Natl. Sci. Acad. USA*, 2004,**101**, 15052.

Keywords: glutathione, transition-state analog, coupling reaction

P.04.02.66

Acta Cryst. (2005). A**61**, C195

Structures of Mnk-2 Reveal Novel Aspects of Kinase Regulation

Ralf Jauch[a], Stefan Jäkel[b], Catharina Netter[c], Kay Schreiter[b], Babette Aicher[b], Herbert Jäckle[a], Markus Wahl[c], [a]*Abteilung für Molekulare Entwicklungsbiologie, Göttingen, Germany.* [c]*Abteilung Zelluläre Biochemie, Max-Planck Institut für biophysikalische Chemie, Göttingen, Germany.* [b]*Develogen AG, Göttingen, Germany.* E-mail: rjauch1@gwdg.de

Human MAP kinase interacting kinase-2 (Mnk-2) targets the translational machinery by phosphorylation of the eukaryotic initiation factor 4e (eIF4E) and plays intricate roles in growth control. Here we present the 2.0 Å crystal structure of the non-phosphorylated Mnk-2 catalytic domain. The results show unique Mnk-specific features such as a zinc binding motif and an atypical open conformation of the activation segment. In addition, the ATP-binding pocket contains Asp-Phe-Asp (DFD) in place of the canonical magnesium-binding Asp-Phe-Gly (DFG) motif. The DFD motif sterically inhibits productive ATP binding as observed with inhibitor-bound p38 kinase. Replacement of DFD by the canonical DFG motif affects the conformation of Mnk-2, but not the ATP-binding and the activity profile of Mnk-2. The results suggest that the ATP binding pocket and the activation segment of Mnk-2 require conformational switches to provide kinase activity.

Keywords: protein kinase, drug design, enzyme regulation

P.04.02.67

Acta Cryst. (2005). A**61**, C195

Tracking X-ray Derived Redox Changes Using Single Crystal Microspectrophotometry

Arwen R. Pearson, Teresa De la Mora Rey, Kevin T. Watts, Ed Hoeffner, Carrie M. Wilmot, *University of Minnesota, Dept. of Biochem., Mol. Biol. & Biophys., Minneapolis, MN 55455, U.S.A.* E-mail: pears079@umn.edu

Methylamine dehydrogenase (MADH) contains a novel quinone cofactor, TTQ, derived from two modified tryptophan residues. MADH catalyses the oxidation of methylamine, with concomitant reduction of TTQ. To complete the catalytic cycle, TTQ is reoxidized by two electron transfer (ET) events.

In the *P. denitrificans* enzyme, the first ET partner is amicyanin, a blue-copper protein. A stable MADH-amicyanin catalytically competent complex can be crystallized, and the structure has been solved [1].

TTQ and copper have spectral features that change during turnover to reflect electron distribution in the complex. Using single crystal visible microspectrophotometry (SCVM) and freeze trapping, catalytic intermediates of MADH in complex with amicyanin have been trapped in the crystalline state. However, the redox-state of these intermediates is extremely sensitive to X-radiation and changes during data collection. We have used in-line SCVM to monitor the redox state of these crystals during data collection at BioCARS (14BM-C). This information enables us to generate composite datasets of each intermediate before radiation induced decay occurs.

[1] Chen L. *et al.*, *Biochemistry*, 1992, **31** 4959.

Keywords: radiation damage, single-crystal spectroscopy, quinoproteins

P.04.02.68

Acta Cryst. (2005). A**61**, C195-C196

Crystal Structures of Two Domains of Bifunctional Enzyme: Human PAPS Synthetase

Nikolina Sekulic[a], Kristen Dietrich[a], Ingo Paarmann[b], Manfried Konrad[b], Arnon Lavie[a], [a]*University of Illinois at Chicago, Department of Biochemistry and Molecular Genetics, Chicago, Illinois 60607, USA.* [b]*Max-Planck Institute for Biophysical Chemistry, Department of Molecular Genetics, Am Fassberg 11, 37077 Gottingen, Germany*. E-mail: nsekul1@uic.edu

PAPS synthetase is the sole enzyme that catalyzes synthesis of PAPS (3'phospoadenosine 5'-phosphosulfatate), which is the ultimate donor of sulfate in higher organisms. PAPS synthesis is a two-step process. In the first step, APS (adenosine 5'-phosphosulfate) is generated from inorganic sulfate and ATP. In the second reaction, APS is phosphorylated on 3'-OH of its sugar ring to yield PAPS molecule. In the lower organisms, these reactions are catalyzed by two