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Structure of γ-glutamylcysteine Synthetase Complexed with Buthionine Sulfoximine

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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

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γ-Glutamylcysteine Synthetase: Peptide Formation Coupling with ATP Hydrolysis

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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

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Structures of Mnk-2 Reveal Novel Aspects of Kinase Regulation

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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

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Tracking X-ray Derived Redox Changes Using Single Crystal Microspectrophotometry

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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

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

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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

separate enzymes ATP sulfurylase and APS kinase, respectively. In humans both activities are present on single polypeptide chain giving bifunctional PAPS synthetase. We have done extensive structural analysis to understand what are the implications of having both activities on one polypeptide chain. Is product of first reaction directly transferred to the active site of the second reaction? Are the two reactions coordinated? If so, how two active sites communicate?

Here we report 2.1 Å crystal structures of ATP sulfurylase domain of human PAPS synthetase in complex with its product APS refined to R_{work}=18% and R_{free}=21%; 2.1 Å crystal structure of APS kinase domain of human PAPS synthetase in complex with its substrate APS refined to R_{work}=25% and R_{free}=29% and another 1.9 Å crystal structure of the same domain in complex with its products PAPS and ADP refined to R_{work}=20% and R_{free}=24%. Our structures do not support an enclosed channel between the two active sites but allow for communication between the domains induced by conformational changes upon substrate binding and catalysis. In addition, our structures explain strong inhibitory effect of APS on APS kinase.

Keywords: PAPS synthetase, ATP sulfurylase, APS kinase

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Structures of Arg-181 Mutant and wild Type of L-lactate Oxidase from *A. viridans*

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L-lactate oxidase (LOX) from *Aerococcus viridans* is a member of the α-hydroxyacid-oxidsase flavoenzyme family and catalyzes the oxidation of L-lactate by O_2 with pyruvate and H_2O_2 as products. Arg-181 residue is conserved throughout the family and located around the FMN. Arg-181 has been replaced by Met to determine the effect of removing the positive charge on the residue. Effects of the replacement on kinetic properties have been reported previously. In this report, there are small effects on reactivity of the reduced flavin with O_2. On the other hand, the efficiency of reduction of the oxidized flavin by L-lactate is greatly reduced. The result demonstrated the importance of Arg-181 residue in the binding substrate and its interaction with flavin. Both Wild type and R181M mutant were crystallized to belong to the tetragonal space group *I*422. We solved both structures about 2 Å resolution using molecular replacement method with glycolate oxidase as a search model, which is a member of the family and 35% identity with LOX. LOX structures formed the tetramer by four-fold symmetry in the asymmetric unit and packed densely in the unit cell. We demonstrate the comparison of structures with other families and the function of Arg-181 residue based on the crystallographic study of LOX structures of Arg-181 mutant and wild type.

Keywords: protein crystallography, flavoprotein, structure-activity relationships of enzymes

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Structure of Small GTPase Human Rheb Provides a Structural Basis for its Unique Biological Function and Reveals a Novel GTP Hydrolysis Mechanism

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Small GTPase Rheb functions as an important mediator between the tumor suppressor proteins TSC1/TSC2 and the mTOR to stimulate cell growth and possesses unique biological features different from other small GTPases. Structures of human Rheb in complexes with GDP, GTP, and GppNHp reveal unique structural features that provide molecular basis for these distinct properties. During GTP/GDP cycling, switch I undergoes conformational change whereas switch II maintains a stable, unusual extended conformation. The unique switch II conformation results in a displacement of Gln64, making it incapable of participating in GTP hydrolysis and thus accounting for the low intrinsic GTPase activity of Rheb. This rearrangement also creates space to accommodate the side chain of Arg15, avoiding the steric conflict with the catalytic residue and explaining its noninvolvement in GTP hydrolysis. A closed GTP-binding site appears to prohibit the insertion of a potential arginine finger from its GAP. Taken the genetics, biochemical, biological, and structural data together, we propose that Rheb forms a new group of the Ras/Rap subfamily and uses a novel GTP hydrolysis mechanism.

Keywords: small GTPase, Rheb, mTOR signaling

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Crystal Structure of Alkyl Hydroperoxide-reductase (AhpC) from *Helicobacter pylori*

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The AhpC protein from *H. pylori*, a thioredoxin (Trx)-dependent alkyl hydroperoxide-reductase, is a member of the ubiquitous 2-Cys peroxiredoxins family (2-Cys Prxs), a group of thiol-specific antioxidant enzymes. Prxs exert the protective antioxidant role in cells through their peroxidase activity, whereby hydrogen peroxide, peroxynitrite and a wide range of organic hydroperoxides (ROOH) are reduced and detoxified ($ROOH + 2e^- \rightarrow ROH + H_2O$).

In this study AhpC has been cloned and overexpressed in *E. coli*. After purification to homogeneity, crystals of the recombinant protein have been grown by hanging-drop vapour diffusion technique. A native data set from a frozen crystal has been collected to 2.95 Å resolution using synchrotron radiation. The crystal structure of AhpC, in the oxidized state, has been determined using the molecular replacement method (R = 23.6%, R_{free} = 25.9 %). The model, similar to other members of the 2-Cys Prx family crystallized as toroide-shaped complexes [1], consists of a pentameric arrangement of homodimers. Oligomerization properties of AhpC have been also characterized. The molecule aggregates giving several oligomeric states in function of salt concentration and redox state, with high ionic strength and oxidized state clearly favouring the decameric assembly.

[1] Wood Z.A., Schroder E., Harris J.R., Poole L.B., *TRENDS in Biochemical Sciences*, 2003, **28**, 32-40.

Keywords: alkyl hydroperoxide-reductase, peroxiredoxin, oxidized decamer

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A Representation of a Possible Intermediate Step during Substrate Recognition of HIV-1 Protease: Crystal Structures of Substrate Bound Enzyme Exhibiting a Novel Flap Conformation

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HIV-1 protease processes and cleaves the Gag and Pol polyproteins allowing viral maturation and is therefore a prime target of anti-viral therapy. In this study, we are reporting two crystal structures of HIV-1 protease (at 1.85 and 1.5Å), complexed to two variants of nucleocapsid-p1 (NC-p1) substrate, where one of the flaps in each complex is found to be in a relatively open conformation in comparison to the canonical liganded flap-closed conformation. The NC-p1 cleavage site is the slowest and rate determining step in the processing of Gag polyprotein. These structures may represent an intermediate step in substrate recognition in HIV protease and their structures will be compared in detail with similar substrate complexes where the flaps are completely closed that we have previously published.

Keywords: HIV, substrate recognition, enzyme