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

P.04.02.69

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

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

Yasufumi Umena[a,b], Kazuko Yorita[c], Takeshi Matsuoka[d], Makoto Abe[a], Akiko Kita[ae], Kiyoshi Fukui[c], Tomitake Tukihara[b], Yukio Morimoto[a,e], [a]*Research Reactor Inst., Kyoto Univ., Japan.* [b]*Inst. for Protein Res., Japan. Osaka Univ., Japan.* [c]*Inst. Enzyme Res., Univ. of Tokushima, Japan.* [d]*Asahi Kasei Pharma,* [e]*RIKEN Harima Inst., Japan.* E-mail: de74yas@rri.kyoto-u.ac.jp

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 Arg-181 has been replaced by Met to determine the effect of removing the positive charge on the residue.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

P.04.02.70

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

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

Jianping Ding, Yadong Yu, Sheng Li, Xiang Xu, *Key Laboratory of Proteomics, Institute of Biochemistry and Cell Biology, SIBS, CAS, China.* E-mail: jpding@sibs.ac.cn

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

P.04.02.71

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

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

Elena Papinutto[a], Henry J. Windle[b], Roberto Battistutta[a], Dermot Kelleher[b], Giuseppe Zanotti[a], [a]*Department of Chemical Sciences, University of Padua, and VIMM, Padua.* [b]*Department of Clinical Medicine, Trinity College, Dublin.* E-mail: elena.papinutto@unipd.it

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

P.04.02.72

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

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

Moses Prabu-Jeyabalan, Ellen A. Nalivaika, Celia A. Schiffer, *Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, Massachusetts 01605, USA.* E-mail: moses.prabu@umassmed.edu

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