P.04.02.94

Acta Cryst. (2005). A**61**, C202

The *E. coli* PDHc E1 Component Complex with a Reaction Intermediate Analogue

Palaniappa Arjunan[a,b], M. Sax[a], A. Brunskill[a,b], N. Nemeria[c], F. Jordan[c], W.Furey[a,b], [a]*Biocrystallography Laboratory, VA Pittsburgh Healthcare System, University Drive C, Pittsburgh, PA 15240.* [b]*Department of Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.* [c]*Department of Chemistry, Rutgers University, Newark, NJ 07102, USA*. E-mail: arjun@pitt.edu

The thiamin diphosphate (ThDP) dependent E1 component of the pyruvate dehydrogenase multienzyme complex (PDHc) catalyzes the decarboxylation of pyruvate and subsequent acetyl transfer to a lipoyl-lysine residue from the E2 component. Biochemical studies of the H407A E1 variant clearly indicated the importance of this residue to the overall reaction of the multienzyme complex. The specific activity of this variant is only 0.15% with respect to the native enzyme. In the native E1 crystal structure the loop region containing the residue H407 was unobserved due to disorder. Superposition of the E1 component and yeast transketolase (TK) structures indicates a general structural similarity and it is clear that if this region becomes ordered as in TK, the H407 residue can come very close to the ThDP and can interact with substrate or reaction intermediates. The crystal structures of the native and H407A variant of *E. coli* PDHc E1, both with a reaction intermediate analogue in its active site, have been determined to a resolution of 2.1 and 1.85Å respectively. Comparison of these two structures clearly indicates that the presence of the substrate analogue in the active site induces conformational changes in its vicinity.

Keywords: thiamin diphosphate, pyruvate dehydrogenase, E1 component

P.04.02.95

Acta Cryst. (2005). A**61**, C202

Crystal Structure of Chitin Binding Domain of Chitinase A1

Takanori Matsuura[a], Izumi Yabuta[a], Tohru Yamaguchi[b], Eriko Chikaishi[a], Yuko Nagasaki[c], Masashi Hara[c], Takeshi Watanabe[c], Kenichi Akagi[a], Hideo Akutsu[a], Takahisa Ikegami[a], Atsushi Nakagawa[a], [a]*Inst. Protein Res., Osaka Univ., Japan.* [b]*Discovery Research Laboratories, Shionogi & Co., Ltd.* [c]*Faculty of Agriculture, Niigata Univ., Japan.* E-mail: t-matsuu@protein.osaka-u.ac.jp

The crystal structure of the chitin-binding domain (ChBD) of chitinase A1 (ChiA1), from *B. circulans* WL-12 has been determined. ChiA1 is a glycosidase that hydrolyzes chitin, and ChBD, ranging from Ala^{655} to Gln^{699} located at the C-terminal, binds specifically to insoluble chitin.

The diffraction data of the $ChBD_{ChiA1}$ crystal were collected at BL44XU at SPring-8, Japan, with the resolution of 0.95 Å. The phase was determined by the molecular replacement method using the structure previously determined by NMR as the model.

$ChBD_{ChiA1}$ has a compact and globular structure with the topology of a twisted β-sandwich. The overall topology is similar to that of the cellulose-binding domain (CBD) of *Erwinia chrysanthemi* endoglucanase Z (CBD_{EGZ}). However, $ChBD_{ChiA1}$ lacks the three aromatic residues (Trp^{18}, Trp^{43}, and Tyr^{44} in CBD_{EGZ}), aligned linearly and exposed to the solvent, which interact with cellulose. Mutation studies suggested that the loop region containing Trp^{687} interact with chitin. Moreover, $ChBD_{ChiA1}$ is detached from chitin by decreasing the pH value in solution from 4 to 3, probably because the charge in the side-chain of Glu^{688} is involved in the chitin-binding. Therefore, the binding mechanism of $ChBD_{ChiA1}$ is expected to be different from that proposed for CBDs.

Keywords: atomic resolution crystallography, glycosyl hydrolases, NMR

P.04.02.96

Acta Cryst. (2005). A**61**, C202

Characterization of TenA from *Bacillus subtilis*: A Thiaminase II

Angela Toms, Amy Haas, Tadhg P. Begley, Steven E. Ealick. *Department of Chemistry and Chemical Biology, Ithaca, NY, 14853 USA*. E-mail: at265@cornell.edu

The biosynthesis of thiamin pyrophosphate has been the focus of considerable effort over the past decade; most of the proteins involved in assembly, salvage, transport and degradation have now been identified and in many cases structurally and mechanistically characterized [1]. A conspicuous exception is TenA, which in *B. subtilis* is part of the thiazole biosynthetic operon. TenA is known to be strongly repressed by thiamin [2], suggesting TenA may have a role in thiamin biosynthesis or metabolism. The structure of TenA alone and in complex with 4-amino-2-methyl-5-hydroxymethyl-pyrimidine have been determined to 2.6 Å and 2.5 Å, respectively. It has also been demonstrated that TenA has thiaminase II activity. The TenA structure suggests that the degradation of thiamin by TenA likely proceeds via the same addition-elimination mechanism described for thiaminase I [3]. While the chemical reaction catalyzed by thiaminases is well defined, the biological function is not yet clear. The over expression of TenA in *B. subtilis* results in an increase in the secretion of the degradative enzymes subtilisin, neutral protease and levansucrase [4], providing evidence of a possible relationship between TenA and the Deg proteins. DegS undergoes autophosphorylation in response to an unkown signal. It is tempting to speculate that this unknown signal may in some way be due to the binding and/or degradation of thiamin by TenA.

[1] Settembre *et al.*, *Curr. Op. Struct. Biol.*, 2003, **13**, 739-747. [2] Lee *et al.*, *J. Bacteriol.*, 2001, **183**, 7371-7380. [3] Nicewonger *et al.*, *J. Org. Chem.*, 1996, **61**, 4172-4174. [4] Pang *et al.*, *J. Bacteriol.*, 1991, **173**, 46-54.

Keywords: thiamin biosynthesis and degradation, enzyme mechanism, transcriptional activator

P.04.02.97

Acta Cryst. (2005). A**61**, C202

Crystal Structure of Inhibitor-bound Mouse Cytidine Deaminase at 1.5 Å

Aik Hong Teh[a], Masaki Yamamoto[b], Makoto Kimura[b], Isamu Yamaguchi[b], Nobuo Tanaka[a], Takashi Kumasaka[a], [a]*Department of Life Science, Tokyo Institute of Technology.* [b]*RIKEN, Japan.* E-mail: ahongteh@bio.titech.ac.jp

Cytidine deaminase (CDA) catalyses the deamination of cytidine and deoxycytidine to uridine and deoxyuridine. Two types of CDA, dimeric and tetrameric CDAs, have been classified [1,2]. The dimeric CDA has two cysteine and one histidine residues liganding a zinc ion at the active site, whereas the three residues are all cysteine in the tetrameric CDA. Arg56 of the tetrameric CDA from *Bacillus subtilis* partly neutralises the negative charge of the cysteine [2,3].

The inhibitor-bound structure of the tetrameric mouse CDA has, surprisingly, revealed the corresponding residue, Arg68, in two alternate conformations. While in the first conformation Arg68 forms hydrogen bonds with two of the zinc-binding cysteine residues, in the second conformation these hydrogen bonds are abolished. Although hydrogen bonds are important for maintaining zinc reactivity [3], the absence of it in the second conformation, conversely, can facilitate product dissociation by increasing negative charge donation from cysteine to the zinc ion, hence weakening the zinc–product interaction. Furthermore, the nearby Gln72 dyad, formed by Gln72 from two adjacent subunits, interacts with Arg68 in the second conformation, suggesting an allosteric cooperativity between the two subunits.

[1] Betts L., Xiang S., Short S.A., Wolfenden R., Carter C.W.J., *J. Mol. Biol.*, 1994, **235**, 635. [2] Johansson E., Mejlhede N., Neuhard J., Larsen S., *Biochemistry*, 2002, **41**, 2563. [3] Johansson E., Neuhard J., Willemoës M., Larsen S., 2004, **43**, 6020.

Keywords: cytidine deaminase, alternate conformation, product dissociation

P.04.02.98

Acta Cryst. (2005). A**61**, C202-C203

***Arabidopsis thaliana* Acyl-CoA Oxidase 1 in Complex with AcetoAcetyl-CoA**

Jenny Berglund, Lise Pedersen, Anette Henriksen, *Department of Chemistry, Carlsberg Laboratory, Valby, Denmark.* E-mail:

jebe@crc.dk

Fatty acid breakdown is an essential process in all organisms, and the complex pathway require several sets of enzymes working in different cellular compartments. β-oxidation cycles are the center of the fatty acid breakdown, in each round the lipid chain shortening by a C_2 unit. The acyl-CoA oxidase family catalyzes the first and rate limiting step in the perixosomal β-oxidation cycle, where acyl-CoA is converted to *trans*-2-enoyl-CoA. *Arabidopsis thaliana* have 4 different acyl-CoA oxidases, each with different chain length specificities. The structure of acyl-CoA oxidase 1 (ACX1), specific for long chain lipids, has previously been determined in our group.

We here present ACX1 in complex with acetoacetyl-CoA; the first structure of an acyl-CoA oxidase in complex with a substrate analogue. The fatty acyl moiety could be modeled between the isoalloxazine ring of FAD and the putative catalytic residue Glu424, forming hydrogen bonds to the backbone nitrogen of Glu424 and to N5 of FAD. Glu424 has moved to better accomodate the inhibitor, and is also more ordered in the complex structure. This confirms Glu424 to be the catalytic residue. The beginning of helix G and the end of helix H has moved slightly, and an interesting rotamer change of His374 and Tyr278 can be observed, bringing both residues closer to the CoA tail of the modeled ligand.

Keywords: acylCoA oxidase, beta oxidation, peroxisome

P.04.02.99

Acta Cryst. (2005). A**61**, C203

Structural Studies on Sulfurtransferase/Phosphatase Enzymes

Domenico Bordo[a], Andrea Spallarossa[a,b], Silvia Pagani[c], Timoty Larson, [d] Martino Bolognesi[b], [a]*National Cancer Research Insitute, Genova.* [b]*University of Genova.* [c]*University of Milano.* [d]*Virginia University*. E-mail: bordo@fisica.unige.it

Sulfurtransferases are widespread enzymes that *in vitro* catalyse the transfer of a sulfur atom from a donor molecule to cyanide [1]. In order to elucidate the molecular basis of sulfur transfer reaction and identify the structural determinants for enzyme selectivity, crystallographic analyses were carried out on three different sulfurtransferases: *Azotobacter vinelandii* rhodanese (RhdA) and *Escherichia coli* GlpE and *Escherichia coli* SseA, a MST enzyme.

The crystal structure of the RhdA has been determined with the method of Multiple Isomorphous Replacement and refined at 1.8 Å resolution in the sulfur-free and persulfide-containing forms [2]. GlpE crystal structure has been determined at 1.06 Å and displays a three-dimensional fold similar to that of either RhdA domains [3]. Notably, GlpE is also structurally similar to the catalytic domain of the human cell cycle-control Cdc25 phosphatase. The distinct substrate specificity, sulphur for rhodanese enzymes and phosphate for Cdc25 phosphatases, appears to be primarily consequence of the different active site loop length in the two enzymes. These structural findings provide guidelines for the identification of the as yet unknown biological role of this protein. Also the crystal structure of SseA, solved at 2.8 Å resolution by molecular replacement method [4].

[1] Bordo D., Bork P., *EMBO Reports*, 2002, **3**, 741. [2] Bordo D. *et al.*, *J. Mol. Biol*., 2000, **398**, 691. [3] Spallarossa A., *et al*., *Structure*, 2001, **9**, 1117. [4] Spallarossa A., *et al*., *J. Mol. Biol.*, 2004, **335**, 583.

Keywords: sulfurtransferases, phosphatases, rhodanese

P.04.02.100

Acta Cryst. (2005). A**61**, C203

Regulation of *Sulfolobus solfataricus* Uracil Phosphoribosyl-transferase

Anders Kadziola[a], Stig Christoffersen[b], Eva Johansson[a], Susan Arent[a], Sine Larsen[a], Kaj Frank Jensen[b], [a]*Department of Chemistry and* [b]*Institute of Molecular Biology and Physiology, University of Copenhagen, Denmark.* E-mail: anders@ccs.ki.ku.dk

UPRTase is a salvage enzyme that catalyzes the formation of UMP from PRPP (5-phosphoribosyl-1-α-diphosphate) and uracil.

CTP and UMP can independently bind to *Ss*UPRTase and simultaneous binding of CTP and UMP strongly inhibits the enzyme. A structure to 2.8 Å resolution of *Ss*UPRTase-CTP-UMP has already been determined [1].

GTP causes a 20-fold increase in the turnover number k_{cat} and raises K_M for PRPP and uracil by 2- and >10-fold, respectively [2]. In order to make an *Ss*UPRTase-GTP-PRPP complex, the enzyme must be depleted for the product UMP. Co-purified UMP binds so strongly to *Ss*UPRTase that an unfolding and refolding procedure was necessary to remove it. UMP-depleted *Ss*UPRTase (5 mg/mL) with 5 mM GTP and 5 mM PRPP was crystallized by vapour diffusion with PEG8000 at pH 6.5. Synchrotron data to 2.8 Å resolution has been recorded ($P6_422$, a=b=122.2 Å, c=62.2 Å) and the structure determined by molecular replacement.

*Ss*UPRTase in solution as well as in crystals is tetrameric with 222 symmetry. The allosteric binding sites for CTP/GTP are situated in the middle of the tetramer ca. 24 Å from the active sites. Transformation from inhibited to activated structure involves structural changes in the quaternary structure along with major active site movements.

[1] Arent S., Harris P., Jensen K.F., Larsen S., *Biochemistry*, 2005, **44**, 883. [2] Jensen K.F., Arent S., Larsen S., Schack L., *FEBS Journal*, 2005, **272**, 1440.

Keywords: nucleotide metabolism, protein regulation, enzyme catalysis

P.04.02.101

Acta Cryst. (2005). A**61**, C203

Crystal Structure of Glutathione-dependent Dehydroascorbate Reductase from Spinach Chloroplast

Tomonori Yadani[a], Shinichi Kurata[a], Hiroyoshi Matsumura[a], Tsuyoshi Inoue[a], Eiichi Mizohata[a], Taise Shimaoka[b], Chikahiro Miyake[b], Akiho Yokota[b], Yasushi Kai[a], [a]*Department of Materials Chemistry, Graduate School of Engineering, Osaka University, Japan.* [b]*Department of Molecular biology, Graduate School of Biological Science, Nara Institute of Science and Technology (NAIST), Japan.* E-mail: tomo@chem.eng.osaka-u.ac.jp

Glutathione-dependent dehydroascorbate reductase (GSH-DHAR) catalyzes the reduction of dehydroascorbate (DHA) to ascorbate using reduced glutathione as the electron donor. GSH-DHAR existing in chloroplast plays a pivotal role in the regeneration of ascorbate, which is oxidized to scavenge active oxygen species in the process of photosynthesis. The catalytic mechanism of the GSH-DHAR from spinach chloroplast is intriguing, because the specific constants for DHA and GSH are much higher than those of the other characterized DHARs.

Here, we report the three-dimensional structure of GSH-DHAR from spinach chloroplasts at 1.65 Å resolution, which has been determined by the multiwavelength anomalous dispersion (MAD) phasing method. The crystal structure reveals a monomeric form, which corresponds to the results observed in the analyses of gel filtration and dynamic light scattering. The structure is mainly composed of the similar domain to that of previously solved thioltransferase [1] and an extra alpha-helical domain. The catalytically essential cystein was completely reduced, because it was crystallized in the solution including high concentration of DTT. The model study using the coordinates of glutathione transferases suggested that the putative glutathione binding site was formed by the amino residues corresponding to those of the other glutathione transferases. These observations support that glutathione could be bound near the catalytically essential cystein. We will show the detailed reaction mechanism to describe how it establishes its high specificities.

[1] Katti S. K., *et al*., *Protein Sci*., 1995, **4**, 1998.

Keywords: crystal structure, dehydroascorbate, reductase

P.04.02.102

Acta Cryst. (2005). A**61**, C203-C204

Snapshots Along the PEPCK Catalytic Pathway

Todd Holyoak, *Department of Biochemistry and Molecular Biology, The University of Kansas Medical Center, Kansas City, Kansas.* E-mail: tholyoak@KUMC.edu

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the reversible decarboxylation of oxaloacetic acid with the concomitant