

[1] Li R., Klinman J.P., Mathews F.S., *Structure*, 1998, **6**(3), 293.

Keywords: copper enzymes, oxygen, activation

P.04.03.6

Acta Cryst. (2005). A61, C210

Structure of Thi1: Thiamin Biosynthesis in *Arabidopsis thaliana*

Glaucius Oliva^a, Paulo H.C. Godoi^a, Marie A. Van-Sluyts^b, Carlos F. M. Menck^c, ^a*Institute of Physics of Sao Carlos, University of São Paulo, Brazil.* ^b*Institute of Biosciences, University of São Paulo, Brazil.* ^c*Institute of Biomedical Sciences, University of São Paulo, Brazil.* E-mail: oliva@ifsc.usp.br

Thiamin is an essential coenzyme in all living organisms. It is formed in two biosynthetic routes, one for the synthesis of 2-methyl-4-amino-5-hydroxymethylpyrimidine pyrophosphate (the pyrimidine moiety) and another for the synthesis of 4-methyl-5-(*b*-hydroxyethyl)thiazole phosphate (the thiazole moiety). Here we describe the three-dimensional structure of Thi1, the only known thiazole biosynthetic enzyme in Eukaryotes, bound to 2-carboxylate-4-methyl-5-*b*-(ethyl adenosine 5'-diphosphate) thiazole. This intermediate has not been described before and was either formed by the enzyme during the Thi1 heterologous expression in *E. coli* or was sequestered from the bacterial cytoplasm. Based on the structure, we putatively propose that Thi1 is the thiazole synthase from *Arabidopsis thaliana*

Crystals grow as thin plates (0.1 x 0.1 x 0.01 mm) by vapor diffusion with well solution of 100 mM MES pH 6.0, 40% (v/v) MPD and 1.5% (w/v) heptane-1,2,3-triol. Crystals belong to space group F222 with unit cell dimensions of *a*=102.356 Å, *b*=133.147 Å and *c*=142.301 Å with two molecules in the asymmetric unit. X-ray fluorescence scan at beamline ID29 of ESRF revealed an unforeseen bound Zn ion which allowed the structure solution by SAD phasing. The final refinement to 1.6 Å includes the one Zn ion and one ligand per monomer and a total of 438 water molecules, with final Rfactor 13.9% and Rfree 17.1%.

Keywords: thiamin biosynthesis, SAD, protein structure

P.04.03.7

Acta Cryst. (2005). A61, C210

Analysis of Mutants of an Active Site Base in a Non-heme Extradial Dioxygenase

Rebecca D. Hoefl, Stephanie L. Groce, John D. Lipscomb, Douglas H. Ohlendorf, *Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota.* E-mail: siem0027@umn.edu

Homoprotocatechuate 2,3-dioxygenase (HPCD) from the Gram-positive soil bacterium *Brevibacterium fuscum* is an extradiol dioxygenase that catalyzes the ring cleavage of 3,4-dihydroxyphenylacetate to α -OH- δ -carboxymethyl *cis,cis*-muconic semialdehyde by insertion of both atoms of molecular oxygen into the ring. HPCD is an Fe²⁺ containing, colorless enzyme that has shown very high substrate cleavage fidelity. One of the residues thought to provide some of this specificity is the highly conserved H200 [1]. In the current mechanistic model, H200 acts as an active site base to activate substrate for oxygen addition [2]. A series of mutations at this site have been created and, to date, three of these mutants H200N, H200Y and H200F have been crystallized. H200Y is red in color; H200N kinetic data reveals an oxygenated intermediate not seen in wild type enzyme; and H200F has been shown to switch from extradiol cleavage to intradiol cleavage of an alternate substrate [1]. Data from these mutants and their complexes are currently being collected and analyzed. Insights into the molecular mechanism resulting from this analysis will be presented.

[1] Groce S.L., Lipscomb J.D., *JACS*, 2003, 11780. [2] Vetting M.W., Wackett L.P., Que L., Lipscomb J.D., Ohlendorf D.H., *Journal of Bacteriology*, 2004, 1945.

Keywords: enzymatic structure-activity relationships, enzyme mechanism kinetics, metalloenzymes

P.04.03.8

Acta Cryst. (2005). A61, C210

Anion-independent Iron Binding by *Campylobacter jejuni* Ferric Binding Protein

Stacey A. L. Tom-Yew, Diana T. Cui, Elena G. Bekker, Michael E. P. Murphy, *Department of Microbiology and Immunology, University of British Columbia, Vancouver BC, Canada.* E-mail: tomyew@interchange.ubc.ca

The bacterium *Campylobacter jejuni* is a leading cause of human gastroenteritis. Under iron limited conditions, *C. jejuni* expresses a ferric binding protein (cFbpA) that in many pathogenic bacteria functions to acquire iron as part of their virulence repertoire. The cFbpA crystal grew in space group P2₁ with 2 molecules in the asymmetric unit. The biological unit is the monomer.

The overall structure of cFbpA [1] consists of 2 globular domains linked by 2 β -strands. The cFbpA crystal structure reveals unprecedented iron coordination in a distorted octahedral geometry by only 5 protein ligands. The histidine and 1 tyrosine are from the N-terminal domain, whereas the 3 remaining tyrosine ligands are from the C-terminal domain. Surprisingly, a synergistic anion is not observed in the cFbpA iron binding site suggesting a novel role for this protein in iron uptake. In the well-characterized ferric transport proteins, the respective synergistic anions are important for iron binding and release. The 4 Tyr ligands are 1.9 to 2.1 Å from the iron and have B-factors similar to that of the iron (13 Å²). In contrast, His14 forms a weaker interaction with iron (~2.3 Å) and the imidazole ring average B-factor is elevated (20.6 Å²). His14 may mediate iron release similar to carbonate in transferrin.

[1] Tom-Yew S.A.L., Cui D. T., Bekker E.G., Murphy M.E.P., *J. Biol. Chem.*, 2004.

Keywords: ion transport, metalloprotein structures, synergistic anion

P.04.03.9

Acta Cryst. (2005). A61, C210

Tat System of *Escherichia coli*: Zn²⁺-bound Structures of tatD, ycfH and yjv

Vladimir N. Malashkevich^a, Dao Feng^b, Frank M. Raushel^b, Steven C. Almo^a, ^a*Department of Biochemistry, Albert Einstein College of Medicine, Bronx, USA.* ^b*Department of Chemistry, Texas A&M University, College Station, USA.* E-mail: vladimir@medusa.aecom.yu.edu

The *Escherichia coli* Tat system mediates Sec-independent export of protein precursors bearing twin arginine signal peptides. Genes known to be involved in this process include *tatA*, *tatB*, and *tatC* that form an operon with a fourth gene, *tatD*. The *tatD* gene product has two homologues in *E. coli* coded by the unlinked *ycfH* and *yjv* genes. The actual role of these enzymes and their substrates are not yet known, however, it was suggested that they might possess Zn²⁺-dependent amidohydrolase activity. Significant number of amidohydrolases share TIM-barrel fold. The diversity of the catalytic mechanisms and substrate specificities is achieved through sequence and structural variation within the loop area. As a part of large-scale genomic effort to establish structure-function relationships within the amidohydrolase family, we determined high-resolution X-ray structures of *tatD*, *ycfH* and *yjv*. Despite relatively low sequence identity of 24-29%, all three structures share similar overall fold, however, the number of Zn²⁺ ions in the active sites and their coordination differ significantly in three enzymes. Despite proposed deoxyribonuclease activity for *tatD*, none of the structures demonstrates nucleotide binding when co-crystallized with short DNA fragment. The potential functional roles of these enzymes will be discussed in the light of the structural and scarce biochemical data.

[1] Wexler M., Sargent F., Jack R.L., Stanley, R.L., Bogsch E.G., Robinson K., Berks B.C., Palmer T., *J. Biol. Chem.*, 2000, **275**, 16717.

Keywords: amidohydrolases, Zn²⁺-enzymes, *tatD*