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Keywords: copper enzymes, oxygen, activation

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Structure of Thi1: Thiamin Biosynthesis in *Arabdopsis thaliana*
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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=142.301Å 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

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Analysis of Mutants of an Active Site Base in a Non-heme Extradiol Dioxygenase
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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 Fe^{2+} 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.

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

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Anion-independent Iron Binding by *Campylobacter jejuni* Ferric Binding Protein
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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 $P2_1$ 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 $Å^2$). In contrast, His14 forms a weaker interaction with iron (~2.3 Å) and the imidazole ring average B-factor is elevated (20.6 $Å^2$). His14 may mediate iron release similar to carbonate in transferrin.

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Keywords: ion transport, metalloprotein structures, synergistic anion

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Tat System of *Escherichia coli*: Zn^{2+}-bound Structures of tatD, ycfH and yjjV
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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 *yjjV* genes. The actual role of these enzymes and their substrates are not yet known, however, it was suggested that they might posses Zn^{2+}-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 yjjV. Despite relatively low sequence identity of 24-29%, all three structures share similar overall fold, however, the number of Zn^{2+} 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.

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Keywords: amidohydrolases, Zn^{2+}-enzymes, tatD