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Substrate Specificity of Three New Intradiol Dioxygenases: an X-ray Characterization

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The crystal structures of Hydroxyquinol 1,2-dioxygenase (1,2-HQD) from Nocardioides simplex 3E [1] and of 3- [2] and 4-chlorocatechol 1,2-dioxygenases [3,4] from the Gram-positive bacterium Rhodococcus opacus 1CP, three Fe(III) ion containing enzymes involved in the aerobic biodegradation of different chloroaromatic compounds, have been recently solved.

The analysis of the structures and their comparison with the catechol 1,2-dioxygenase from Acinetobacter calcoaceticus ADP1 (1,2-CTD), highlights significant differences between these enzymes. The active site cavities present several dissimilarities, with respect to the known catechol cleaving enzyme, suggesting the key-role of specific amino-acidic residues in substrate selection. A co-crystallized benzoate- or hydroxamate-like ions, were found bound to the metal center of the three enzymes and revealed details on novel modes of inhibitors binding. The 1,2-HQD structure show one of the most distinctive characteristics among all intradiol dioxygenases; two extensive openings and the consequent exposure to solvent of the upper part of the catalytic cavity are arranged to favor the binding of hydroxyquinols but not catechols. Among the amino acid residues expected to interact with substrates that are different from the corresponding analogues of 1,2-CTD, a few were selected as responsible for the observed substrate selectivity differences in the three distinctive enzymes.

[1] Ferraroni M., Seifert J., Travkin V.M., Thiel M., Kaschabek S., Scozzafava A., Golovleva L., Schlöman M., Briganti F., *J. Biol. Chem.*, 2005, *in press*. [2] Ferraroni M., Ruiz Tarifa M.Y., Scozzafava A., Solyanikova I.P., Kolomytseva M.P., Golovleva L., Briganti F., *Acta Cryst.*, 2003, **D59**, 188-190. [3] Ferraroni M., Ruiz Tarifa M.Y., Briganti F., Scozzafava A., Mangani S., Solyanikova I.P., Kolomytseva M.P., Golovleva L., *Acta Cryst.*, 2002, **D58**, 1074-1076. [4] Ferraroni M., Solyanikova I.P., Kolomytseva M.P., Scozzafava A., Golovleva L., Briganti F., *J. Biol. Chem.*, 2004, **279**, 27646-27655.

Keywords: enzyme specificity, metalloenzymes, non-heme iron protein

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Crystal Structure of Iron Superoxide Dismutase from Obligate Anaerobic Bacterium

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Superoxide dismutase scavenges the superoxide radical (O_2^-) to form molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) and forms part of the defense mechanism of cells against free radical oxidative damages. We identified the iron superoxide dismutase from obligate anaerobic bacterium *Desulfovibrio vulgaris* Miyazaki F and constructed an expression system in *Escherichia coli* [1]. Crystallization was carried out using hanging drop vapor diffusion method with PEG6000 (space group $P2_1$; a=51.96 Å, b=83.07 Å, c=61.16 Å, â=114.5 ^{o}). The crystal structure has been determined by molecular replacement and refined to 1.0 Å resolution. The crystallographic R and free R are 17.9% and 19.2%, respectively. There are two identical monomers in the asymmetric unit. The monomer has a molecular weight of 22 kDa and consists of 205 amino acid residues of which 201 are visible in the electron density map. The overall fold of the monomer of *D. vulgaris* Fe-SOD is similar to that of other known Fe/Mn-SODs. The active site is composed of one iron, four metal ligand residues (His34, His84, Asp170 and His174) and one water molecule. The interaction of the dimer interface is also similar to that of other Fe/Mn-SODs. The structure differences compared with other Fe/Mn-SODs are at the loop regions on the surface of the molecule (Asp68-Ala72 and Gly143-Asp145).

[1] Nakanishi T., et. al., *J. Biochemistry*, 2003, **133**, 387-393.

Keywords: superoxide dismutase, iron, crystal structure

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Understanding how the Alzheimer's Amyloid Presursor Protein binds Copper Ions

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Alzheimer's disease is a debilitating neurodegenerative disorder. Soluble oligomers of Aβ peptides are neurotoxic and thought to trigger the development of the disease. The interaction between copper (Cu) ions and the transmembrane amyloid precursor protein (APP) in the brain may play a key role in modulating the pathogenesis. The binding of extracellular Cu^{2+} to APP in vitro lowers the processing of APP into Aβ When Cu^{2+} is supplemented in their diet, transgenic mice over-producing Aβ had improved survival and decreased soluble Aβ level. However, the administration of significant amounts of Cu^{2+} in humans is likely to cause toxic side-effects. Structural studies of Cu^{2+} binding to APP will therefore aid development of suitable Cu^{2+} mimetics for use in treating the disease.

The APP interacts with and reduces Cu^{2+} ions through the extra-cellular copper binding domain (CuBD). The crystal structure of CuBD in metal-free (apo) form is determined to 0.85 Å resolution using X-ray diffraction data at a synchrotron. The structure of CuBD bound with Cu^{2+} was obtained from apo crystals soaked in a solution containing $CuCl_2$, and a pursuant reduction step generated the Cu^+-bound structure. In both cases, the Cu ion is coordinated to His147, His151, Tyr168 and a water molecule, in a distorted square planar geometry. As the water ligand might represent an amino acid ligand from another APP domain or between APP molecules, X-ray absorption spectroscopy of Cu binding in solution is being pursued while longer APP constructs covering the CuBD are studied and crystallised.

Keywords: macromolecular crystallography, metal ions in biology, Alzheimer's proteins

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Dioxygen Activation in *Hansenula polymorpha* Amine Oxidase

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Copper amine oxidases (CAO) are homodimeric enzymes that convert primary amines to aldehydes and O_2 to H_2O_2. Each monomer contains a Cu(II) ion and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor. O_2 is key in the oxidative-half reaction of CAO, returning the substrate reduced aminoquinol TPQ back to the oxidized quinone state. However, the exact location and timepoint of O_2 binding in the oxidative half-reaction remains unclear.

The crystal structure of oxidized wild type *H. polymorpha* amine oxidase (wtHPAO) was solved previously [1]. In this study, gas binding is observed in wtHPAO as well as mutants with altered O_2 activation kinetics. Xe can be used to map hydrophobic sites in proteins where molecular O_2 may bind. CO and NO are oxygen mimics used extensively in solution studies to probe dioxygen activation. These gases are complexed to substrate reduced wtHPAO anaerobically in the crystal. The resulting structures give insight into O_2 binding and activation. In addition, parallel structural studies of O_2 binding mutants provide insight into the specific amino acids that play a role in directing and assisting O_2 binding.

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

[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

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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.

[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

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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.

[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, Zn^{2+}-enzymes, tatD