

P.04.03.2*Acta Cryst.* (2005). A61, C209**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

P.04.03.3*Acta Cryst.* (2005). A61, C209**Crystal Structure of Iron Superoxide Dismutase from Obligate Anaerobic Bacterium**

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Superoxide dismutase scavenges the superoxide radical (O₂⁻) to form molecular oxygen (O₂) and hydrogen peroxide (H₂O₂) 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 P2₁; a=51.96 Å, b=83.07 Å, c=61.16 Å, β =114.5°). 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

P.04.03.4*Acta Cryst.* (2005). A61, C209**Understanding how the Alzheimer's Amyloid Precursor Protein binds Copper Ions**

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Alzheimer's disease is a debilitating neurodegenerative disorder. Soluble oligomers of A β 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 Cu²⁺ to APP in vitro lowers the processing of APP into A β . When Cu²⁺ is supplemented in their diet, transgenic mice over-producing A β had improved survival and decreased soluble A β level. However, the administration of significant amounts of Cu²⁺ in humans is likely to cause toxic side-effects. Structural studies of Cu²⁺ binding to APP will therefore aid development of suitable Cu²⁺ mimetics for use in treating the disease.

The APP interacts with and reduces Cu²⁺ ions through the extracellular 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 Cu²⁺ was obtained from apo crystals soaked in a solution containing CuCl₂, 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

P.04.03.5*Acta Cryst.* (2005). A61, C209-C210**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 O₂ to H₂O₂. Each monomer contains a Cu(II) ion and a 2,4,5-trihydroxyphenylalanine quinone (TPQ) cofactor. O₂ 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 O₂ 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 O₂ activation kinetics. Xe can be used to map hydrophobic sites in proteins where molecular O₂ 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 O₂ binding and activation. In addition, parallel structural studies of O₂ binding mutants provide insight into the specific amino acids that play a role in directing and assisting O₂ binding.