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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.

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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.