AN UNUSUAL CYS-TYR COVALENT BOND PRESENT IN A LARGE CATALASE

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Catalase-1 (Cat1) of *Neurospora crassa* was crystallized by the hanging drop method and a crystal was diffracted at the Stanford Synchrotron Radiation Laboratory. X-ray reflection data were analyzed with denzo, xdisp and CCP4 programs. The crystal belongs to the space group C2, having unit cell parameters of a = 130.007, b = 182.242, c = 90.364 Å, β = 133.413° and a dimer in the asymmetric unit. Cat1 structure was determined at 1.75 A resolution using the molecular replacement method and a *Escherichia coli* catalase (HPII) as starting model. The final R and Rfree values were 18.64 and 20.29, respectively. Cat1 is a large catalase with a C-terminal flavodoxin-like domain, similar in structure to the HPII. Heme in Cat1 was a mixture of protoheme IX (57%) and a heme with two hydroxyl groups in ring D, one of which formed a spirolactone with the propionyl group (43%). HPII also has a heme with a spirolactone. In HPII, the Tyr that coordinates the heme Fe(III) proximally makes a covalent bond with a neighbor His. Interestingly, the equivalent Tyr in Cat-1 makes a covalent bond with a Cys, instead. The corresponding bond being formed between Cys 356 sulfur and Tyr 379 Cb.
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Keywords: CATALASE STRUCTURE FUNCTION RELATIONSHIPS NEUROSPORA CRASSA

***MYCOBACTERIUM TUBERCULOSIS THYMIDYLATE* KINASE: STRUCTURAL STUDIES OF THE ENZYMATIC REACTION**

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Thymidine and thymidylate kinases are ubiquitous enzymes essential to DNA synthesis and cell replication. They are suitable targets for the rational development of new drugs. With 2 millions deaths every year, tuberculosis is the primary cause of mortality among infectious agents. Early biochemical and structural characterisation has suggested that *Mycobacterium tuberculosis thymidilate* kinase (Mt-tmpk) shares properties of both eucaryotic and procaryotic enzymes. Therefore, in an effort to identify new targets against the disease, we set out to study the catalytic mechanism of Mt-tmpk. We have obtained a new crystal form of the complex Mt-tmpk/dTMP (deoxythymidine-5'-monophosphate) by using sodium malonate. In one monomer (A) dTMP is observed and the LID, a flexible stretch that covers the phosphoryl donor binding site, is in a partially closed conformation. In the other monomer (B), a magnesium ion coordinates dTMP and the LID is held tighter. By soaking crystals with ATP (adenosine-5'-triphosphate), turnover was initiated. In monomer A, the reaction was 65 percent complete and the products were released, leaving an empty structure with the LID in a fully open configuration. In monomer B, the complex with dTDP, ADP and magnesium was observed and the LID is closed. These data explain the induced-fit mechanism by which ATP binding is promoted by dTMP binding through LID closure. The presence of magnesium coordinating the phosphoryl acceptor is a unique feature of Mt-tmpk. By soaking crystals with ATP at acidic pH, we observed no formation of dTDP and no magnesium. We concluded that magnesium was indispensable for catalysis.

Keywords: **THYMIDYLATE-KINASE, CATALYTIC-MECHANISM, pH**

THE X-RAY STRUCTURE OF YEAST 5-AMINOLAEVULINIC ACID DEHYDRATASE (ALAD) COMPLEXED WITH SUBSTRATE AND INHIBITORS

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ALAD produces the pyrrole porphobilinogen (PBG). The enzyme is a homo-octamer of TIM barrel subunits. Each subunit joins two molecules of 5-aminolaevulinic acid (ALA) to form PBG. Four PBG molecules are cyclized by subsequent enzymes ultimately to produce tetrapyrroles such as haem and chlorophyll. Hereditary defects in ALAD cause Doss porphyria and the enzyme is sensitive to inhibition by metals such as lead and mercury, which disrupt a triple cysteine zinc cofactor site. ALAD is also inhibited potently by succinylacetone which is produced in large amounts by tyrosinaemia (typeI) patients.
The structures of yeast ALAD complexed with substrate (ALA) and various inhibitors including laevulinic acid (LA), succinylacetone (SA) and 4-keto-5-aminolaevulinic acid (KAH) have been solved at high resolution. The ligands all bind by forming a covalent Schiff base link with Lys263 at the active site. These structures define the interactions made by one of the two substrate molecules (P-side). SA binding is notable because it alone induces a conformational change, KAH binding because it appears to form a stable carbinolamine intermediate with Lys263.
Yeast ALAD complexed with an irreversible inhibitor 4,7-dioxosebacic acid has also been solved. This structure defines interactions made by both of the substrate molecules (A- and P-side) and shows that a second Schiff base can be formed at the active site between ligand and Lys210. A catalytic mechanism involving both substrates linked to the enzyme through Schiff bases is thus envisaged. A substrate complex in which an inter-substrate Schiff base has formed is also described.

Keywords: **CATALYTIC MECHANISM, SUBSTRATE BINDING, TETRAPYRROLE BIOSYNTHESIS**

EVOLVING AN ENZYME INTO DIFFERENT METABOLIC ROLES: STRUCTURES OF FUNGAL AND BACTERIAL ATP SULFURYLASE

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ATP sulfurylase is a ubiquitous enzyme found in all taxa of life. In eukaryotes and heterotropic bacteria it catalyzes the reaction between ATP and sulfate to produce APS and pyrophosphate. This is the first step in the biological assimilation of inorganic sulfate, which is proceeded by reactions catalyzed by either APS reductase or APS kinase and ultimately gives rise to all of the sulfur containing molecules synthesized by these organisms. However, chemoautotrophic bacteria use ATP sulfurylase to catalyze the opposite direction of the reaction, to produce ATP from APS and pyrophosphate. Such bacteria typically live in environments with high concentrations of reduced sulfur compounds and have an energy metabolism based on the oxidation of hydrogen sulfide. The final step in the overall oxidation of hydrogen sulfide to inorganic sulfate is catalyzed by ATP sulfurylase, which may be the sole substrate level phosphorylation in the energy-producing pathway of these bacteria. ATP sulfurylase has been evolved and optimized in each of these organisms to meet their different metabolic needs. The crystal structure of ATP sulfurylase from both organisms has been determined and has revealed the residues that interact with the substrates and catalyze the reaction are invariant. However, the structures also reveal that a nearby loop may constitute a 'second layer' of active site residues, which may modulate the differences in kinetic properties required for the enzymes different metabolic roles.

Keywords: **ATP SULFURYLASE, SULFATE, EVOLUTION**