PS04.02.23 METAL COORDINATION OF SEVERAL DIVALENT CATIONS: Mg²⁺, Ca²⁺, Be²⁺, AND Zn²⁺.

A conformational change occurs when the crystals are soaked with 3-iodotyrosine, a clinically prescribed inhibitor of TH. The soaked crystals have two monomers in the asymmetric unit. The new space group is C222₁ with unit cell dimensions of a=72.6Å, b=154.1Å, c=155.5Å. Data have been collected from the inhibitor-bound form to 2.6Å resolution also at SSRL beamline 7-1.

Multiple isomorphous replacement is being used to determine the structures for both space groups (derivatives - Hg, Ph, Au, and selene-methionine). Several good derivative data sets have been collected both with a rotating anode source and at SSRL.

Data is also being collected for a complex containing all participants in the reaction - iron, porin and substrate or substrate analog. Additionally, a physiologically relevant complex containing the feedback inhibitor dopamine is also being studied.

We would like to thank our collaborator in this project, Paul Fitzpatrick from Texas A&M University.

PS04.02.24 STRUCTURAL STUDIES OF TYROSINE HYDROXYLASE IN THE APO-ENZYME AND INHIBITOR-BOUND STATES.

The DNA repair enzyme endonuclease IV (endoIV), which is induced by superoxide, catalyzes the cleavage of DNA at apurinic/apyrimidinic (abasic or AP) sites resulting from reactive oxygen damage. It is critical for the survival of pathogens in the presence of host superoxide-mediated defenses. To understand the mechanism of damaged DNA detection and cleavage, we are solving structure of endoIV from E. coli and Mycobacterium leprae. These endoIV enzymes were crystallized in triclinic and monoclinic crystal forms. A 2.4Å resolution native data set from the monoclinic crystal form (space group P2₁) with the unit cell dimensions of a=49Å, b=60Å, and c=51Å has been collected at 180°C with flash cooling cryogenic device using MAR image plate area detector at Siemens rotating anode generator. We are now scanning crystals soaked in heavy atom compounds in order to find isomorphous derivatives to complete this new structure determination and current results on these structures will be presented.

Manganese superoxide dismutase (MnSOD) protects mitochondria against superoxide-mediated oxidative damage. MnSOD has usually high stability and fast catalysis. The structure of the native protein was previously solved at 2.2Å resolution and Tyr 34 was proposed to serve as a proton carrier during the catalysis. To address the role of Tyr 34, the crystal structure of Y34F mutant MnSOD has been solved in two different crystal forms (P6₃22 and P212121) using the molecular replacement method in the AMoRE program package. The structure for the hexagonal crystal form was refined to 1.9Å resolution with the R-factor of 19% using the diffraction data collected at the UCSD Research Resource for Protein Crystallography. The orthorhombic form structure was refined to 2.0Å resolution. Similar to the wildtype MnSOD, the crystal structure of the mutant is a homotetramer with Ph34 located in the active site. Each subunit is composed of the N-terminal helical hairpin domain and the C-terminal оfо domains. Both domains contribute ligands to the catalytic manganese site. Current structural implications for MnSOD stability and activity will be presented.

Tyrosine hydroxylase (TH) is the rate limiting step in aromatic amino acid hydroxylases. These hydroxylases require iron and biotin as cofactors to add one atom from molecular oxygen to the aromatic ring. To date, no crystal structures have been solved in this family of enzymes.

The catalytic domain of rat TH has been cloned and expressed (Daubner, S.C. et al.1993. Protein Science 2, 1452-1460). This protein is as catalytically active as the whole enzyme. Both holo-TH and the catalytic domain have been shown to form a tetramer in solution.

Crystals of this enzyme have been grown from ammonium sulfate. Data for the apo-enzyme have been collected to a resolution of 2.3Å at SSRL beamline 7-1. The space group is F222 with unit cell dimensions of a=59.3Å, b=151.5Å, c=192.7Å. There is one monomer in the asymmetric unit.