PS04.02.23 METAL COORDINATION OF SEVERAL DIVALENT CATIONS: Mg^{2+},Ca^{2+},Be^{2+}, AND Zn^{2+}. Jenny P. Glusker†, Amy Kaufman Katz†, Charles W. Bock‡, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA (†), The Philadelphia College of Textiles and Science, Philadelphia, Pennsylvania 19144, USA (‡)

We have examined the coordination preferences of the divalent ions Mg^{2+}, Ca^{2+}, Be^{2+}, and Zn^{2+} by analyses of crystal structures in the Cambridge Structural Database (CSD)[1], the Protein Databank (PDB)[2], and *ab initio* molecular orbital calculations that provide energetics data on the binding of water molecules to these cations.

Our findings are that magnesium has a firm coordination number of 6 with an overwhelming preference for water or other oxygen-containing ligands.[3] Beryllium[4] has the same preference for oxygen, but with a coordination number of 4. The energy consequences of changing the coordination number of Mg^{2+} from 6 to any other number are high. Zinc ions, with the same size charge, are softer, and also bind sulfur, and nitrogen in addition to oxygen. Their coordination number can readily vary between 4 and 6 with little energetic penalty. Therefore zinc is better able than magnesium to mediate chemical reactions in enzymes.[5] Calcium ions appear to bind with a higher coordination number and values of 7-9 are observed in crystal structures. Oxygen is the preferred ligand. There is not much energetic change between Ca^{2+} with 7 or 8 bound water molecules.[6]

In protein crystal structures magnesium ions tend to bind two water molecules (ideal for displacement by a binding substrate in an enzyme), while calcium more often binds only one water molecule. The geometry of binding of enzymatic carboxylate groups is also different between calcium and magnesium in that calcium may share both oxygen atoms of the carboxylate group while magnesium only binds one. The results of these surveys will be illustrated.

Supported by grant CA-10925 from National Institutes of Health.

1. Allen, F. H. et al *Acta Cryst.* **1979**, *B35*, 2331-2339.
2. Bernstein, F. C. et al. *J. Molec. Biol.* **1977**,112, *535-542.*
3. Bock, C. W, Kaufman, A, Glusker, J. P *Inorg. Chem.* **1994**, *33*, 419-427.
4. Bock, C. W., Glusker, J. P. *Inorg. Chem.* **1993**, *32*, 1242-1250.
5. Bock, C. W., Katz, A. K. Glusker, J. P. *J. Amer. Chem. Soc.* **1995**,117, 3754-3765.
6. Katz A. K., Bock,C. W., Glusker J. P. *J. Amer. Chem. Soc.* (in press)

PS04.02.24 STRUCTURAL STUDIES OF TYROSINE HYDROXYLASE IN THE APO-ENZYME AND INHIBITOR-BOUND STATES. Kenneth E.Goodwill*, Cara B. Marks, Christelle Sabatier, & Raymond C. Stevens, Chemistry Department, University of California, Berkeley 94720

Tyrosine hydroxylase (TH) is the rate limiting step in catecholamine (dopamine, adrenaline, and noradrenaline) biosynthesis. It is a member of the closely homologous family of aromatic amino acid hydroxylases. These hydroxylases require iron and biopterin 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.

A conformational change occurs when the crytals 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 $C222_1$ 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, Pb, Au, and seleno-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, pterin and substrate or substrate analog. Additionally, a physiologically relevant complex containing the feed-back inhibitor dopamine is also being studied.

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

PS04.02.25 STRUCTURAL CHARACTERIZATION OF REACTIVE OXYGEN DEFENCE ENZYMES: THE ENDONUCLEASE IV AND Y34F MUTANT MN SUPEROXIDE DISMUTASE. Yue Guan[1], Katrina Forest[1], Gloria Borgstahl[1], Michael Hickey[1], Richard Cunningham[2], John A. Tainer[1], [1]The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037, [2]SUNY at Albany, Albany, NY 12222

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 2.4-Å resolution native data set from the monoclinic crystal form (space group $P2_1$ 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 scaning 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 2.2-Å 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 ($P6_122$ and $P2_12_12$) using the molecular replacement method in the AMoRe program package. The structure for the hexagonal crystal form was refined to 1.9-Å 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 2.0-Å resolution. Similar to the wildtype MnSOD, the crystal structure of the mutant is a homotetramer with Phe34 located in the active site. Each subunit is composed of the N-terminal helical hairpin domain and the C-terminal α/β domains. Both domains contribute ligands to the catalytic manganese site. Current structural implications for MnSOD stability and activity will be presented