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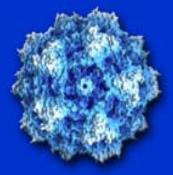

Proposed Neutron Diffraction Studies to Elucidate the Proton Transfer Mechanism via Hydrogen Bonded Networks in Carbonic Anhydrase and Superoxide Dismutase.

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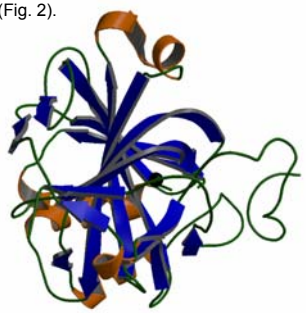
Carbonic Anhydrase

Carbonic anhydrase (CA) catalyzes the hydration of carbon dioxide and the dehydration of bicarbonate: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. The mammalian α-carbonic anhydrases (α-CA) and α-CA-domains in more complex isoforms consist of a single polypeptide chain (unmodified molecular weight ~ 29 kD) that function as monomers with one Zn^{2+} ion (Fig. 1). The functions of α-CA are diverse and include; renal and male reproductive duct acidification, modulation of hemoglobin's affinity for O_2 in respiration, acid/base balance, gluconeogenesis (supplies HCO_3^- to pyruvate carboxylase for glucose production), ureagenesis (supplies HCO_3^- to carbamoyl phosphate synthetase for urea production), ion transport/regulation (Na^+/H^+ and Cl^-/HCO_3^- exchange), and gastric acid production. Catalysis by CA is limited in maximal velocity by proton transfer (10^6 s^{-1}) between the active site zinc-bound water and residue H64, that functions as a proton acceptor/donor in the shuttling pathway to and from the bulk solvent (B) of the environment and the zinc-bound water (Fig. 2).



$$CO_2 + EZnOH^- \leftrightarrow EZnHCO_3^- \xrightarrow{H_2O} EZnH_2O + HCO_3^-$$

$$EZnH_2O + B \leftrightarrow EZnOH^- + BH^+$$

Fig.1. Ribbon diagram of HCA II. The Zn atom is shown as a black sphere.

Proton transfer between the active site zinc-bound water and residue H64 proceeds through intervening solvent molecules spanning a distance of ~7 Å. Extensive X-ray crystallography, kinetics, and site-specific mutagenesis experiments have been performed in an attempt to understand the role of hydrogen bonding in the water network in the proton transfer process (Fig. 2).

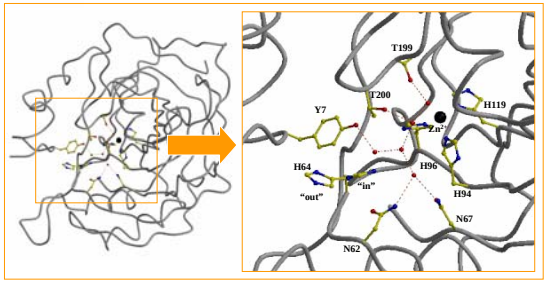


Fig.2. Proposed hydrogen bonding network of HCA II.

Superoxide Dismutase

Manganese superoxide dismutase (MnSOD) catalyzes the dismutation of two molecules of superoxide anion into water and hydrogen peroxide: $2O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$. Human MnSOD is a homotetramer with one Mn^{3+} ion per monomer (Fig. 3). MnSOD scavenges superoxide anions, the highly reactive oxygen species generated by univalent reduction of molecular oxygen during cellular respiration, O_2 radicals are damaging to cellular constituents because they attack proteins, nucleic acids and membrane lipids, thereby disrupting cellular function and integrity. The cumulative effect of this cellular damage contributes to many cellular pathologies including; mutagenesis, carcinogenesis, diabetes, neurodegenerative disease, inflammatory diseases, as well as to the overall process of cellular aging. Catalysis by MnSOD has a rapid (10^4 s^{-1}) proton transfer rate in catalysis. However, in contrast to carbonic anhydrase where several water molecules are utilized, the hydrogen bonded network involves up to five amino-acid side chains and two intervening water molecules (Fig. 3).

$$Mn(III)SOD + O_2^{\cdot-} \rightarrow Mn(III)SOD\text{-}O_2^{\cdot-} \rightarrow Mn(II)SOD + O_2$$

$$Mn(II)SOD + O_2^{\cdot-} + 2H^+ \rightarrow Mn(II)SOD\text{-}O_2^{\cdot-} + 2H^+ \rightarrow Mn(III)SOD + H_2O_2$$

Fig.3. Ribbon diagram of tetrameric MnSOD. Each monomer is a different color and Mn atoms are shown as pink spheres.

X-ray crystallography studies have been insufficient in the elucidation of the extent of this network. Kinetic data shows that the integrity of the entire hydrogen bonded network is essential for maximal velocity and that the disruption of the network at any site decreases the efficiency of catalysis (Fig. 4).

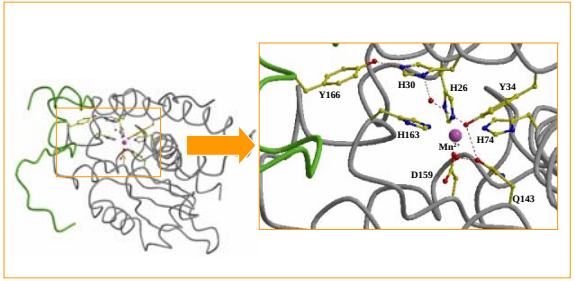


Fig.4. Proposed hydrogen bonding network of MnSOD.

Preliminary Data

In preparation for neutron diffraction studies the following preliminary data have/are been obtained:

Fully perdeuterated HCA II has been synthesized and purified at ILL-EMBL. Monoclinic ($P2_1$) crystals (0.4 x 0.4 x 0.1 mm, Fig. 5) have been obtained (ammonium sulphate 2.75 M, $HgCl_2$ 1 mM, Tris-HCl 100 mM pH 7.8) with unit cell dimensions a = 42.1, b = 41.4, c = 71.9 Å, and β = 103.9°. The X-ray diffraction quality of hydrogenated HCA II crystals growing under these conditions are excellent and diffract to 1.0 Å resolution at ESRF.

Human MnSOD is in the process of being synthesized under fully deuterated conditions at Oakridge. Hydrogenated MnSOD orthorhombic ($P2_12_12_1$) crystals (1.0 x 0.5 x 0.3 mm) have been obtained (25% Polyethylene Glycol 3350, 25 mM potassium phosphate pH 7.6) with unit cell dimensions a = 73.2, b= 77.5, c = 135.2 Å. The X-ray diffraction quality of these crystals are good and diffract to 2.0 Å resolution on an in-house source.

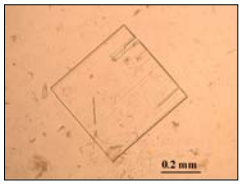


Fig.5. Crystals of fully perdeuterated HCA II.

Expectation of Studies

The expected space group, size, and diffraction quality of fully perdeuterated HCA II and MnSOD crystals should of sufficient quality to be suitable for Neutron diffraction studies at Oakridge/ILL EMBO. Neutron diffraction studies are possibly the only practical method to provide the unambiguous structure analysis required to elucidate the role of hydrogen bonded chains in the rapid intramolecular proton within these two biologically important protein environments.

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