The Allosteric Role of Zinc in Lou Gehrig's Disease

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The degenerative motor neurological disease Amyotrophic Lateral Sclerosis (ALS), commonly called Lou Gehrig's Disease, presently affects about one in 10,000 Americans.[1] Symptoms manifest after age 40 and include loss of motor control, difficulty speaking, muscle cramping and eventual paralysis, with fatality typically within 5 years.[2,3] A majority of patients display Sporadic (random) ALS, while only 10% or patients exhibit the genetically linked Familial ALS. Approximately 25% of the Familial ALS patients show mutations in the cytosolic superoxide scavenger Cu/Zn superoxide dismutase (Cu/Zn SOD).[2] This represents an important point for developing a molecular-level understanding of ALS.

Cu/Zn SOD exists *in vivo* as a 31.2 kDa homo-dimer, with each subunit containing both a copper binding site and a zinc binding site.[4] The copper ion binds to 4 histidine residues (His^{46}, His^{48}, His^{63}, and His^{120}) and one solvent H_2O in a distorted square pyramidal geometry.[4] The zinc ion binds to 3 histidine residues (His^{63}, His^{71}, His^{80}) and one carboxylic oxygen (Asp^{83}). Histidine 63 forms an imidazolate bridge between the copper(II) and zinc(II) (Figure 1).[4]

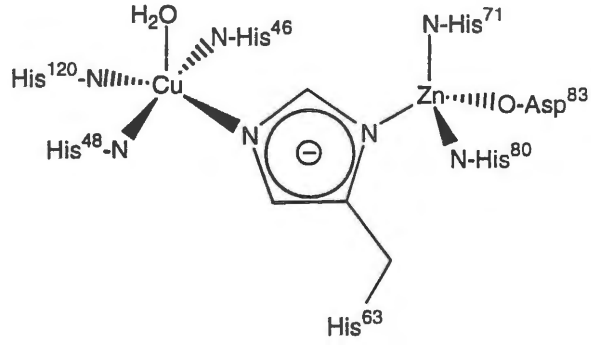

Figure 1

The one electron reduction of molecular oxygen yields a potentially hazardous superoxide anion (O_2^-). Ubiquitous throughout nature, Cu/Zn SOD catalyzes the disproportion reaction of superoxide into hydrogen peroxide and molecular oxygen:[5,6]

$$O_2^- + Cu^{II}Zn^{II}SOD \longrightarrow O_2 + Cu^{I}Zn^{II}SOD$$

$$O_2^- + 2H^+ + Cu^{I}Zn^{II}SOD \longrightarrow H_2O_2 + Cu^{II}Zn^{II}SOD$$

This very efficient reaction proceeds at near diffusion control limits, 2 x 10^9 M^{-1} s^{-1}.[5,6] The entering superoxide reduces the copper(II) to copper(I). In conjunction with two protons, a second superoxide anion reoxidizes the copper(I) via a hydroperoxo intermediate.[7] The imidazolate-zinc bridge has an allosteric effect on the reactivity of the copper site. ALS mutations affect the binding of zinc, which in turn affects the reactivity of the copper center.[5]

To aid in the study of this bimetallic active site, a variety of model complexes have been synthesized.[7,8] In the 1970's Lippard and co-workers[8] synthesized a series of imidazolate bridged dicopper complexes, making the first major contributions to this emerging field. Using electron paramagnetic spectroscopy and magnetic susceptibility, Lippard and coworkers studied interactions across the imidazolate bridge.[8]

Fukuzumi and co-workers[7,9] have recently synthesized a stable imidazolate bridged Cu/Zn model complex. This novel complex exhibits the highest SOD activity of any model complex to date.[7] In addition to high activity levels, this complex has shown spectroscopic evidence for the intermediate species.[10] Using a semiquinone radical anion as a model substrate, they presented evidence for the zinc ion activating the copper ion center by causing a positive shift in the reduction potential.

In addition to model complex analysis, extensive *in vitro* studies have been made on Cu/Zn SOD and its relation to ALS.[5,6,11] Many studies have focused on the enzyme activity and metal binding properties of ALS mutant Cu/Zn SOD as compared to wild type Cu/Zn SOD. It has been shown by Gurney and co-workers[12] that some strains of ALS mutant Cu/Zn SOD have the same superoxide dismutase activity as wild type. It has also been shown that addition of wild type Cu/Zn SOD to ALS mutant Cu/Zn SOD does not protect motor neurons *in vitro*.[1,13] This has led current thought to focus on a new and toxic gain-of-function of ALS mutant Cu/Zn SOD. Three hypotheses have been proposed as to the nature of this gain-of-function[6]: (1) an increased ability of ALS Cu/Zn SOD to catalyze the formation of hydroxyl radicals[1]; (2) an increased ability to form peroxynitrite[16]; and (3) the induction of neurofilament-L aggregation.[13]

When either wild type or ALS mutant SOD become zinc deficient, increased motor neuron death is observed.[11] ALS mutant SOD has a 18- 30 fold lower binding affinity for zinc, allowing existence in the toxic zinc deficient state for an extended period of time. The binding of zinc hinders copper reactivity by restricting the substrate binding only to the weak binding axial position.[5] Zinc deficient SOD allows the substrate to bind to the stronger bonding equatorial position. This stronger bond causes slow product release, allowing the zinc deficient SOD increased reactivity towards other substrates. The augmented study of this hypothesis is key to a molecular understanding of Cu/Zn SOD and its relation to ALS.

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