INVESTIGATING THE AGGREGATION AND TOXICITY OF APO CU/ZN SUPEROXIDE DISMUTASE 1 IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that results in the progressive degeneration of the upper and lower motor neurons. There is no cure for ALS and the pathogenesis of the disease is currently unknown. Approximately 10% of ALS cases are inherited, and among those cases 20% of patients have a mutation on the gene encoding Cu/Zn superoxide dismutase 1 (SOD). Superoxide dismutase is a homodimeric antioxidant protein with a copper and zinc cofactor per subunit that significantly contributes to its stability. Evidence suggests that metal deficient SOD may be the precursor to the formation of toxic SOD aggregates. To better understand the mechanism by which SOD mutations may lead to ALS, a series of aggregation experiments have been performed using wild type apo (demetallated) SOD under physiological-like conditions. When incubated in a physiological concentration of potassium chloride (150 mM), at 37 ºC and pH 7.4, dimeric apo SOD spontaneously formed tetrameric and higher molecular weight species, including spherical aggregates and annular, pore-like structures. When holo (metallated) SOD was studied under identical conditions, the amount of aggregation observed was significantly less than that of apo SOD and no formation of annular, pore-like structures occurred. When the free cysteine 111 was chemically blocked, aggregation was dramatically reduced, indicating that cysteine 111 may be involved in SOD aggregation. Incubation of apo SOD with cultures of mouse hippocampus cells resulted in significant amounts of cell death. In contrast, holo SOD did not have a toxic effect on mouse hippocampus cells. These results demonstrate that apo wild type SOD can form annular pore-like species and higher-ordered aggregates under physiological-like conditions, suggesting that the loss of metals

within wild type SOD and mutants thereof may be the key pathogenic event that ultimately leads to the development of ALS.