Investigating the Copper Transfer Mechanism between CCS and SOD1: Biophysical Studies on the SOD1-CCS Interaction

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Copper chaperone for superoxide dismutase (CCS) incorporates copper ion with copper/zinc superoxide dismutase (SOD1) in the cell. A key question is how this copper-chaperone delivers $Cu^{1+/2+}$ to target enzyme in the high metal chelating capacity of the cytosol. To elucidate the SOD1-CCS interaction and their copper transfer mechanisms, the human SOD1-CCS system is being evaluated from two aspects: 1) the coordination structure and oxidation state of copper during the transfer process and 2) binding affinity (thermodynamics) of human SOD1-CCS interaction. Several new roles for the copper chaperone in the conversion of the immature SOD1 polypeptide to the active state provide a basis for a deeper understanding of the cause of familial ALS. The implications of these new findings for the treatment of ALS will be discussed.

The crystal structure of the heterodimer between H48F-yeast SOD1 and yeast CCS has already been reported; however, His48 is a copper coordinating ligand, and this mutation prevents copper binding and thus does not provide information on the coordination chemistry of the transfer event. As an alternative way of trapping heterodimer with copper ion, a cysteine mutant of human SOD1 (C6,111,146S) was used because these mutations are expected not to affect copper coordination. Interestingly a new heterodimeric intermediate has been trapped and the coordination chemistry is being investigated by X-ray absorption. We have found that the copper transfer process from CCS to SOD1 requires dioxygen, which ultimately plays a role in maturation of the reduced Cys to the disulfide state and may also oxidize the copper center. Given that the coordination structure switch from Cys-rich (CCS) to His-rich (SOD1) environments, change in oxidation state of copper ion is plausible. The interaction of several post-translational modification states of SOD1 of CCS will be compared to elucidate the mechanisms of O_2 reduction, disulfide formation and copper transfer steps.

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