

MS03-P20 | THE SOD1-HCCS MECHANISM INVOLVED IN COPPER HOMEOSTASIS

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The copper concentration in cell is maintained at low levels and regulatory processes control its homeostasis. Once soluble Cu^{+1} has been transferred to the cytosol through CTR1, glutathione or recipient proteins immediately bind to the metal and deliver it to its target. Cu,Zn-Superoxide dismutase (SOD1) is one of the proteins which requires copper as a cofactor for enzymatic activity. However, SOD1 is not expected to acquire copper via direct interaction with CTR1. It is well known that hCCS (human copper chaperone for SOD1) forms stable heterodimers with SOD1. This interaction suggests subsequent amendments which involve reorientation of hCCS-D1, copper transfer and oxidation of a disulphide bond. The objective of this work is to investigate the recognition processes and sequence of post translational modifications during the maturation of SOD1 catalyzed by hCCS and particularly if the Cu^{+1} transfer occurs within the context of a membrane scaffold involving CTR1-hCCS or CTR1-hCCS-SOD1 interactions. Crystals of heterodimeric complexes which were either SOD1 copper free or loaded in complex with its metallochaperone were obtained, processed and analyzed. Protein-Membrane association experiments on different combinations of hCCS and SOD1 mutants suggested that copper acquisition occurs via hCCS, in its homodimeric state, engaging with the lipid bilayer and subsequently forming heterodimers off the membrane. Moreover, the analysis of the structures of heterodimeric complexes showed a novel conformation for the SOD1 disulphide sub-loop which communicates the presence of hCCS to the SOD1 active site and coordinates the timing of copper transfers prior to the disulphide bond formation.