Copper(I) binding and transfer by the N-terminus of the Wilson disease protein

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The Wilson disease protein (WD) is a transport ATPase involved in copper homeostasis in the liver, brain, and kidney. Mutations in WD and its homolog, the Menkes protein (MNK), lead to genetic disorders of copper metabolism known as Wilson disease and Menkes syndrome, respectively. The WD and MNK proteins receive copper ions from the metallochaperone Atox1 through direct protein-protein interactions. Both WD and MNK comprise six soluble N-terminal domains (N-WD) each containing a conserved CXXC metal-binding motif, eight transmembrane (TM) helices, a conserved CPC sequence located within the sixth TM helix, and an ATP-binding domain. The exact roles of each of the six N-terminal metal-binding domains are not well established, but possible functions include copper exchange with Atox1 and copper-responsive cellular relocalization. Although all six domains of N-WD can bind copper and are structurally similar, genetic and biochemical studies indicate that the domains are not functionally equivalent. One way the domains could be tuned to perform different functions is by having different affinities either for Atox1 or for Cu(I). In order to study the properties of each domain we have prepared a series of Cys to Ser site-directed mutants of N-WD where only one out of the six metal binding sites was preserved. For each N-WD mutant the Cu(I) binding affinity, ability to receive and donate Cu(I) from and to Atox1 will be presented.