

METAL TRANSFER EXPERIMENTS WITH N-TERMINAL METAL BINDING DOMAINS OF WILSON PROTEIN

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Wilson protein, a copper-transporting P-type ATPase found in the copper secretory pathway in the liver and kidney, contains six cytosolic N-terminal metal binding domains that contain the conserved motif MXCXXC. We have cloned and expressed residues 57-129 and 485-633 of Wilson protein, corresponding to metal-binding domains 1 (WLN1) and 4 (WLN4). High resolution gel filtration experiments calibrated with other metal-binding domains reveal that WLN1 and WLN4 behave as monomers in solution and isoelectric focusing gives pIs of 6.77 and 3.85, respectively. WLN1 and WLN4 both bind one equivalent of copper per protein monomer and measured metal to protein ratios are 0.97 and 0.84, respectively. In order to test whether copper transfer is possible between isolated copper-binding domains we incubated Cu-WLN1 with *apo*-WLN4 anaerobically in 20 mM MES/Na, 150 mM NaCl, pH 6.0, then separated the proteins via anion exchange chromatography using a linear NaCl gradient. In control experiments we demonstrated that WLN1 does not bind to the column HiTrap Q-Sepharose (Amersham Pharmacia) but WLN4 remains strongly bound and elutes after the application of the gradient. We demonstrate *in vitro* copper transfer between these domains irrespective of initial copper donor, suggesting that *in vivo* copper transfer between metal-binding domains is possible.