Copper Chaperones: A Bioinorganic Approach to Understanding Copper Trafficking

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Metal ions are required in biological systems, where they perform a variety of specific functions associated with life processes.[1] Metal ions involved in biological redox catalysis can be detrimental to cells, however, by catalyzing unwanted reactions and modifying proteins and nucleic acids via the formation of hydroxyl radicals.[2,3] Organisms must often balance the level of these metal ions between low levels that would cause deficiency in the cells and high levels that could be toxic; both cases can lead to cellular dysfunctions.[4] Living systems have complex mechanisms for regulation of cellular levels of metal ions (homeostasis) and intracellular metal ion uptake, transport, and placement into enzymes (trafficking).[5] Recently, a new class of proteins, copper chaperones, has been implicated in copper homeostasis and trafficking.

Copper chaperones are a class of proteins found in plant, bacterial, yeast, and animal cells. These proteins are responsible both for copper trafficking and for preventing copper ions in transit from damaging cellular components.[5,6] The majority of the copper chaperones known today contain one or more copies of a metal binding motif consisting of a conserved amino acid sequence.[5-10] Most chaperones, despite their conserved sequences and similar secondary and tertiary structures, are specific for their target protein. This specificity is based on slight structural differences between chaperones.[5,11] Several genetic disorders, including Menkes disease and Wilson disease, involve disruption of copper transport via mutations in the structure of copper chaperone-like proteins.[4,12,13]

Recent studies of model compounds and native structures of several copper chaperone proteins have provided new insight into the mechanisms of copper binding in cellular copper chaperones.[6,7,14,15] No crystal structures of metallochaperones were determined before 1999, so model compounds were used to study the metal binding properties of these proteins. Model compounds of Cu(I) with two or three sulfur ligands and those with 4 nitrogen ligands have been compared spectroscopically with Cu(I) bound to Atx1, a cytoplasmic copper chaperone. X-ray absorption near edge structure (XANES) data[9] (Figure 1) demonstrate that the model compound giving the closest spectrum to that of the native enzyme has copper bound in a trigonal planar fashion to three sulfur atoms. A more recent structural study, however, disputes the three coordinate binding of copper in chaperones and chaperone-like proteins.[16]

Mercury NMR has been used as a copper analogue to determine the coordination environments in several copper proteins.[17,18] The mercury derivative of Atx1[6] (Figure 2) was used to determine the crystal structure. In this structure, the first published crystal structure of a metallochaperone, the mercury is bound by only two sulfur ligands. The copper form of Atx1 was successfully crystallized, but the metal binding loop was consistently disordered, therefore the exact coordination of bound copper in Atx1 remains unresolved.[6]

Despite high binding affinities of most target proteins for copper, copper chaperones are required for the activation of enzymes due to the low intracellular copper concentration, proposed to be less than one "free" copper ion per cell.[10] This suggests the absence a pool of "free" copper ions used in the activation of metalloenzymes. The transfer of the copper from the chaperone to the target protein occurs via juxtaposition of metal binding motifs of the two proteins. This is followed by direct insertion of the copper into the active site.[10] Further

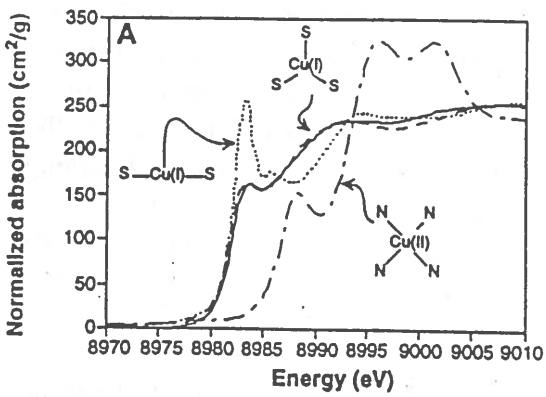

Figure 1

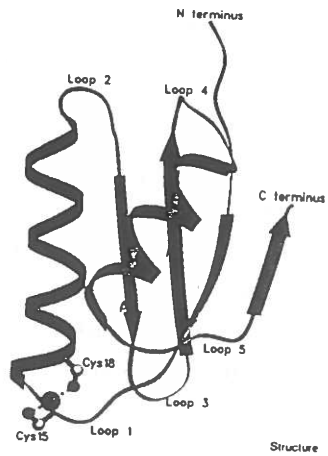

Figure 2

studies of model compounds and crystal structures have aided in understanding the roles of copper chaperones.[7,14]

Although recent progress has been made in understanding copper trafficking, more research is needed before viable gene therapies can be developed to cure copper chaperone related dysfunctions. The similar metal binding regions of other metallochaperones allow the results of the copper chaperone studies to be applied to other metal chaperone systems, such as iron and cobalt chaperones.[5] The results can also be applied to the large class of proteins with tertiary structures resembling the basic structure of most copper chaperones.

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