THE REACTION OF EDTA WITH METALLOTHIONEIN AND ITS SEPARATE CLUSTERS: COMPLEX KINETIC BEHAVIOR WITH NOVEL PROTEIN CONCENTRATION DEPENDENCE

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Mammalian metallothionein (MT) is involved in the detoxification of Cd(II) ions. It binds 7 Cd(II) in two Cd-thiolate clusters (Cd_4S_{11} and Cd_3S_9) which are located in two protein domains. ^{111}Cd and 2-D NMR studies show that in lobster, two Cd_3S_9 domains exist. Despite homologies in the location of cysteines between the corresponding domains of the lobster and mammalian MTs (8 of the 9 cysteines in each domain), the sequence specific Cd-thiolate connections differ in both domains of the lobster protein. To see if these differences alter the chemistry of the proteins, two reactions related to the function of the protein were compared.

Mammalian Cd_7 -MT reacts with EDTA with biphasic kinetics to form Cd-EDTA and apoMT. The derived rate law is consistent with the initial formation of a Cd_7 -MT-EDTA adduct.

$$k_{\text{obs}} = k + \frac{k'[\text{EDTA}]}{K_D + [\text{EDTA}]}$$

Examination of the reactions of isolated α - and β -domains with EDTA show generally similar kinetics, but demonstrate that for the α -domain that cadmium removal proceeds through a partially Cd-depleted intermediate Cd_3S_{11} . According to ^{111}Cd NMR titration of the holo-protein, shows a slight preference for removing Cd from the 3-metal cluster.

The metal exchange between apoMT with Cd-substituted carbonic anhydrase was considerably faster than that involving EDTA, with 30-50% exchange occurring in 30 min. Comparative reactions with lobster MT will also be described. (Supported by NIH grants ES-04026 and ES-4184).