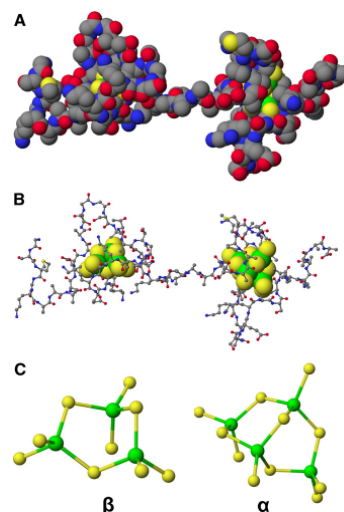


Metalation pathways in metallothionein elucidated with time- and temperature-resolved ESI-mass spectrometry

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Metallothioneins (MTs) are ubiquitous metalloproteins. Spectroscopic probes used for equilibrium studies during the step-wise, metal-by-metal metalation have established that metallothionein binds readily, generally rapidly, and reproducibly a wide range of metals. Indeed, over many years of study, it has been shown that the same CD spectrum is obtained no matter how the product is prepared. Metalation titrations monitored by CD spectroscopy have demonstrated that while end-points can be associated with different “magic” numbers for different metals, the spectroscopic data also indicated that metallothionein can exist with both fewer and more metals than these “magic” numbers. Modeling results of Duncan and Stillman clearly show for all MT peptides studied, that there may be thermodynamically stable conformations for metal-free MT with the cysteine thiols moved to the surface of an essentially globular protein. Time- and temperature-resolved ESI-MS data have provided information on the mechanism of As(III)-metalation. Each of the six As(III) ions bind in a bimolecular, noncooperative manner to human MT1a. The individual rate constants, activation energies and Arrhenius factors were calculated individually for each metal ion bound. A key result when comparing the rates of metallation of the isolated fragments and the two-domain



beta-alpha human metallothionein, is that the trend in rate constants for the two-domain protein can be modeled based on the initial presence of six equivalent binding sites implying that As-metalation of MT follows well known trends in coordination chemistry. This increase in initial rates as a function of possible binding sites may be the key to the evolution of a two-domain MT in higher life forms compared with single-domain MTs in lower life forms as this allows two-domain MTs to bind metals faster, and, as a result, scavenge metals more efficiently.

The talk will end with a discussion of the ramification of

rate constants that decrease significantly as the number of sites available decreases. The trend in rate constants observed for As(III)-metalation of MT helps to explain the observation of the presence of partially metalated MT, that must be the consequence of unoccupied metal binding sites in MT in both in vivo and in vitro experiments. While the initial binding rates are large, the subsequent rate constants decrease proportionally to the number of binding sites remaining, thus the final metal bound will do so at a greatly reduced rate compared to the first rate constant. Given the difficulty involved in binding the last few metals, it is not surprising that partially metalated MT exists.

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