mgr Agnieszka Drozd

Summary

Title: Characteristics of stability and reactivity of human metallothionein isoforms in the context of cellular zinc homeostasis

Human metallothioneins (MTs) are cytoplasmic low molecular weight proteins with high content of cysteine residues responsible for binding metal ions considered as soft and medium according to HSAB theory. First part of studies performed in this thesis addressed biophysical differences among metallothionein homologs: MT1a, MT1b, MT1e, MT1f, MT1g, MT1h, MT1x, MT2a, MT3, and MT4, particularly between seven MT1 isoforms that have been hardly studied in the context of zinc homeostasis before. For this purpose, metallothioneins were overexpressed in bacterial expression system, purified and characterized in terms of biophysical features. Further studies concerned stability and reactivity of these proteins by monitoring Zn(II) ions transfer between metallothioneins and other zinc proteins and peptides. Our results supported the hypothesis of the existence of the weakly bound Zn(II) ion in all MTs. Moreover, we showed that there are thermodynamic differences between MT isoforms. Their stability decreases with the increase of their reactivity, understood as the ability of protein to release Zn(II) ions. Partially saturated species of metallothionein, namely Zn_6MT, Zn_5MT and Zn_4MT, play an important role in Zn(II) ions buffering and in the maintenance of Zn(II) ions homeostasis. In the second part of this thesis we performed cysteine mapping in MT2a to identify the positions of cysteine donors that bind consecutive molar equivalents of Zn(II) ions. For this purpose, we applied chemical labeling of cysteine residues with iodoacetamide and then the trypsin digestion of the labeled protein, followed by separation and identification of tryptic fragments using LC-MS. Based on these results we traced the metalation pathway of MT2a and proposed a model of Zn(II) metalation of MT2a by showing potential structures of MT species partially depleted with Zn2Å. Moreover, we showed that the cysteine donor that occupies the 21st position in the amino acid sequence of MT2a is not involved in Zn(II) binding when the protein is saturated with six molar equivalents of Zn(II). The same conclusion resulted from the Zn(II) dissociation of MT2a performed with apo-form of sorbitol dehydrogenase - zinc enzyme. Thereby, we confirmed by the metalation and demetalation processes of MT2a, that the 21st cysteine donor participates solely in the binding of the seventh Zn(II) ion and

creates the weak Zn(II) binding site in MT2a. The seventh Zn(II) ion, called also the weakly or loosely bound, is the first one to be exchanged between metallothioneins and effector proteins. The weak Zn(II) binding site in MT2a and its role in acceptor-donor properties of metallothioneins is also discussed in this thesis. Separate studies were conducted on the isolated β- and α-domains of MT2a. They revealed that Zn(II) ions bind to isolated domains with an indistinguishable affinity and the lack of weak Zn(II) binding site was observed. Contrary to the popular in the literature opinion, we found that single domain peptides are not good models for studies of entireMT2a protein.

Keywords:
metallothionein, protein isoforms, zinc ions, cadmium ions, zinc proteins, zinc-sulfur cluster, cellular zinc buffering, zinc homeostasis, zinc ions competition, metal ions transfer, metalation, metal-induced folding, cysteine mapping, protein structure