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REVIEW

Metallothioneins and its role in metal regulation, binding of reactive oxygen species, apoptosis and cell differentiation

Bolesław Floriańczyk

Department of Environmental Hygiene, Institute of Agricultural Medicine, Lublin, Poland

Abstract: Metallothioneins (MT) are the widespread proteins in animal world. These proteins are characterized by the low invariability of their structure. The number of aminoacids is fixed in every animal group, that is 60 (or 61) aminoacids, 20 of which are the cysteins radicals what makes over 30% of the aminoacid composition. Such a big ammount of cysteins which include the reactive sulfhydryl groups -SH determinates the metallothionein's functions. Metallothionein take part in the homeostasis of the ions of the metals which are necessary for the proper metabolism of the organism (zinc, copper), and they also take part in the detoxication of the tissue from toxic metals. Apart from these they also protect the tissue from the reactive oxygen species. Metallothioneins are present in high concentration in fast-dividing and transformating cells. Experiments indicate that MT involvement in the process of cell proliferation and differentiation. The presence in MT molecules of a large number of thiolic groups with nucleophilic properties renders them capable of binding not only metallic cathions but also reactive oxygen species and organic radicals (loose electron couples beside a suphur atom easily bind with elements having an electron hole). Numerous studies point out an MT influence on the process of apoptosis.

Key words: metallothionein, metals, apoptosis, reactive oxygen species, cell differentiation

INTRODUCTION

In 1957 a protein responsible for cadmium accumulation was extracted from equine kidney by Margoshes and Vallee. Owing to large amounts of bound metal and the abundance of cysteine residues, the protein was called metallothionein (MT). Further studies revealed that the protein is commonly found in the whole animal kingdom and is synthesized by all tissues of an organism, which points to its universal character [1].

Metallothioneins are low molecular weight proteins of 6-7 kDa. MT molecules contain 20 residues of cysteine, which approximately amounts to 30% of amino acid content. Large amounts of cystein with sulphydril groups determine protein activity [2]. Metallothioneins help maintain the homeostasis of metallic ions that are crucial for undisturbed metabolism (zinc, copper); they regulate the functioning and biosynthesis of zincprotein (such as zinc dependent transcription factors); they take part in detoxication processes [3]; they protect the cell against reactive oxygen species (ROS), ionizing radiation and electrophilic pharmaceuticals used in the treatment of cancer, and from other mutagens [4-8].

Metallothionein synthesis is triggered by many different factors: heavy metals, inflammatory agents, free radicals, glycocorticoids and pharmacological agents [9, 10].

Corresponding author: Bolesław Floriańczyk, MD, Phd, Department of Environmental Hygiene, Institute of Agricultural Medicine, Jaczewskiego 2, 20-090 Lublin, Poland. E-mail: bolkofs@poczta.onet.pl

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STRUCTURE OF METALLOTHIONEINS

Metallothioneins are made up of a single chain and their amino acid composition is quite conservative. Independently of their source, extracted from the organs of different animal species, they only slightly differ between themselves in their amino acid content.

A characteristic feature of an MT chain is the sequence of Cys-X-Cys, Cys-X-Cys or Cys-X-Y-Cys, where X and Y denote an amino acid other than cystein. Studies on metallothioneins reveal a highly conservative pattern in the distribution of cystein regarding its location and sequence.

The number of cysteins is fixed at 20 residues (30% amino acid content). Other amino acids included in the molecule comprise: 6-8 lysine residues, 7-10 serine residues and acetylated methionin as N-final amino acid. There are no aromatic amino acids or histidine.

Cystein is a permanent structural element of matallothioneins in all animal species. The electrophilic character of sulphur in the sulphydril groups of the amino acid is responsible for their high affinity to metallic ions. Metallothioneins display the highest affinity to metals of the transitory groups (e.g. zinc, cadmium, mercury, copper and silver). The bound metal forms tetraedric structures, where 4 cysteine residues take part in co-ordinative metal binding. The affinity to metals is as follows: Ag > Hg > Cu > Cd > Zn > Co = Ni. One molecule of metallothionein can bind 7 atoms of bivalent metals (zinc, cadmium) or a greater number (12 atoms) of univalent ones (e.g. silver) [2].

ABILITY TO BIND METALS

The role of metallothioneins as proteins involved in the metabolism of metals indispensable for growth, development and functioning of an organism is now a fact [11]. MT's are a reservoir of zinc and copper ions (Zn nd Cu). They provide macromolecules requiring zinc and copper with those microelements. MT's also restore proper functions to those metallic enzymes that were previously inactivated by heavy metals. For example, proteins that lost their ability to function as a result of exposition to cadmium, regained those abilities after incubation with zinc metallothionein (ZnMT). Metallothionein is then involved in zinc-cadmium exchange. Thereby, metallothioneins not only bind excessive amounts of metals inside a cell, but also restore the functioning abilities to proteins inactivated by other metals [12].

METALLOTHIONEIN ROLE IN CELL DIFFERENTIATION

Metallothioneins are present in high concentration in fast-dividing and transformating cells. During the process of embrionic development the concentration of metallothioneins is very high then gradually decreases as the thymus shrinks. In adults, MT concentration increases during a regeneration process, e.g. following liver resection. Those experiments indicate MT involvement in the process of cell proliferation and differentiation. Recently discovered molecular forms of MT-3 and MT-4 which are not induced by traditional factors activating metallothioneins Type 1 and Type 2 can be important for cell maturation [13, 14].

METALLOTHIONEIN AND THE ANTIOXIDATIVE SYSTEM

Another suggested function of MT's is their involvement as part of the antioxidative system of the body. The presence in MT molecules of a large number of thiolic groups with nucleophilic properties renders them capable of binding not only metallic cathions but also reactive oxygen species and organic radicals (loose electron couples beside a suphur atom easily bind with elements having an electron hole). The induction of MT synthesis correlates with an increase in the metallic environment of alkylizing agents or free radicals. This is the way in which metallothioneins protect the cell from lethal compounds. The distribution of MT's in the cell allows them to protect all the cell groups against the aforementioned hazards [15].

METALLOTHIONEIN IN THE PROCESS OF APOPTOSIS

Numerous studies point out an MT influence on the process of apoptosis. Studies on cell cultures imply that a higher expression of MT's blocks the process of apoptosis whereas cells deprived of genes for metallothionein witness an increase of it [16, 17]. This is caused by changes in the concentration of intracellular zinc. Other studies using antisense oligonucleotides have shown that a decrease in MT concentration in cells not only blocks the growth of the cell culture but also initiates the process of apoptosis. The mechanism through which metallothioneins block the process of apoptosis has also been discovered. Metallothioneins within the area of the nucleus associate with the p_{50} subunit of transcription factor NF-kappa B, which stabilizes the coupling of that factor with the nuclear DNA [18].

The NF-kappa B has a bicomponent structure: it consists of subunits p_{50} and p_{65}. It resides in the cytoplasm as an inactive complex together with an inhibitory protein (I-kappa B). The alliance with the inhibitor guarantees that the factor remains within the area of the cytoplasm. A vulnerable link in the complex is two cysteine residues included in the p_{50} subunit, which are sensitive to oxidating agents. The oxidation of those residues by e.g. reactive oxygen species (ROS) activates protein kinase, which catalyses the phosphorylation of the inhibitor molecules and its dissociation from the complex. The inhibitor molecule then undergoes ubiquitination and degradation by means of proteolytic enzymes. NF-kappa B transcription factor deprived of the inhibitor molecule translocates through the nuclear membrane. The oxidized form of the factor undergoes further reduction by tioredoxin and then binds to a sequence of nuclear DNA in a mechanism dependent on zinc ions and metallothionein. The factor induces a transcription of many genes and further processes mediate some effects inhibiting apoptosis [18].

Although metallothioneins are deprived of a sequence guaranteeing the translocation to the nucleus (NLS or *nuclear localization signal*), the MT translocation is often described as being a result of co-translocation. However, proteins involved in the process are not yet known [19, 20]. It is interesting that the synthesis of NF-kappa B factor and metallothioneins is triggered by the same factors: TNF, interleukin 1, hypoxia or the reactive forms of oxygen.

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