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REVIEW

Role of Zinc in nervous system cells

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Abstract

Zinc has been identified as one of the most important minerals in the human body. As a trace element, zinc (Zn) is vital for the growth and development of an organism. Its basic functioning centres around its involvement in the structure of macro-elementary compounds, and the activation of numerous enzymes engaged in the metabolic processes. In the organic world, over 300 zinc-dependent enzymes have been identified. Our bodies need zinc to make insulin and eliminate toxins, and zinc is also important for the anti-oxidant maintenance and maintaining a healthy immune system. Laboratory experiments appeared discharged its ant-oxidant functions. The first is that dozens of vital enzymes within the body contain zinc, and in these enzymes the zinc molecule acts directly as an anti-oxidant, protecting the biochemical structure of the enzyme from free radical attack. Secondly, zinc acts to stabilize protein which may otherwise react with highly unstable minerals, particularly iron and copper, to form free radicals. Furthermore, zinc plays a very important role in normal brain development and function. In growing organisms, zinc is known to be indispensable for the undisturbed formation of their nervous systems. In adults, zinc deficiency results in behavioral symptoms, such as memory problems, malaise, or higher susceptibility to stress. On the other hand, it is believed that an excess of free zinc is detrimental and can lead to neuronal death. Studies confirm that the toxicity of zinc shows up when there is an increase in the third fraction or free zinc in a cell (pool of zinc, so called 'free" zinc, which is not bound to proteins). The neurotoxicity of zinc has been demonstrated on animal models in which a stroke, ischemia, Alzheimer's disease, or convulsions were induced. The detailed mechanism of the toxic activity of zinc is not known, but it seems that the main cause of neuronal death is low energy production by mitochondria.

Key words

zinc, nerves system cell, zinc transporting proteins, metallothionein

INTRODUCTION

Zinc (Zn) level control in the cell. A step forward made in the studies of zinc homeostasis was the discovery of protein transporters and metallothioneins – intracellular zinc-binding proteins. Neurons, like most cells, have several transporting proteins at their disposal: those within the membrane, responsible for the uptake and removal of excess zinc, and transporting proteins in the membranes of intracellular organella, responsible for its sequestration. Zinc binding in the cytosol is regulated by metal-binding proteins, among which metallothioneins are the most important elements. They are also unique because of their ability to pass on zinc to other proteins, whether structural and/or enzymatic. The above findings are important as either deficiency or excess of this microelement in the nervous system has serious consequences.

Both zinc depletion and zinc excess are unfavourable for the living organism [1,2]. In order to prevent intracellular zinc from exceeding the critical values, it has to be chelated and its excess removed. Many cells, including neurons, have two ways in which to uptake zinc: carrier-mediated transport, and through voltage-gated canal. Neurons, like most cells, have several transporting proteins at their disposal: those within the membrane, responsible for the uptake and removal of excessive zinc, and transporting proteins in the membranes of intracellular organella, responsible for its sequestration [3-5].

Inside the cell, on the other hand, metallothioneins are the proteins responsible for chelating most of the zinc [7,8].

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Table 1. Brief characteristics of zinc transporting proteins [6]

CDF (cation diffusion facilitator)-**transporting proteins**	These proteins exhibit an unusual degree of sequence divergence and size variation (300-750 residues). CDF proteins possess 6 trans-membrane domains, aminic and carboxylic end, similar to ZIP proteins, directed toward the centre of the cell. On the inside, between trans-membrane domains IV and V, there is a histidine rich region (HRR). Eukaryotic proteins exhibit differences in cell localization and polarity. Thus, some catalyze heavy metal uptake, while others catalyze efflux, some are found in plasma membranes, while others are in organellar membranes. The mechanisms of energy coupling are not well understood, but these proteins are secondary carriers which utilize the pmf, and therefore probably function by H+ antiport (for metal efflux) or H+ symport (for metal uptake).
ZIP family (Zrt-Irt-like) **proteins**	Members of the ZIP family (Fig. 2) consist of 220-430 amino acyl residues with 8 putative trans-membrane spanners. They are derived from animals, plants and yeast, bacteria and archaea. These transporters belonging to the family are located in the membrane, have 8 trans-membrane segments, and their aminic and carboxylic ends are directed toward the centre of the cell. The fragment of the protein molecule responsible for ion binding is situated within the segment between trans-membrane domains III and IV.
DMT (*divalent metal transporters*)	Proteins belonging to this family have 12 trans-membrane domains. The molecular ends (aminic and carboxylic) are directed toward the centre of the cell. The loop joining domains VII and VIII has a glycosylating region. With these proteins, unlike in the previous families, there is no histidine rich region. Despite this, the loop joining domains VIII and IX has an ion-transporting motif.

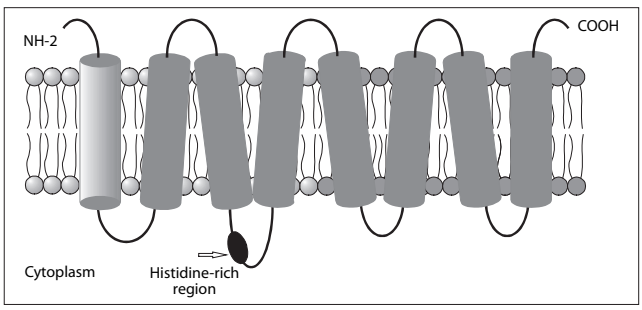

Figure 1. ZIP transporters

Metallothioneins not only bind zinc, but also mediate in passing it on to other proteins (zinc proteins) which require zinc ions to operate, and which co-operate with the transporting proteins within the cell membranes [9].

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 differ slightly 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 (Fig. 1). Studies of 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). Cystein is a permanent structural element of matallothioneins in all animal species. The electrophilic character of sulfur in the sulfhydril groups (HS-) of the amino acid is responsible for their high affinity to metallic ions. Metallothioneins display the highest affinity to zinc. The bound metal forms tetraedric structures, where 4 cystein residues take part in co-ordinative metal binding. One molecule of metallothionein can bind 7 atoms of zinc [10].

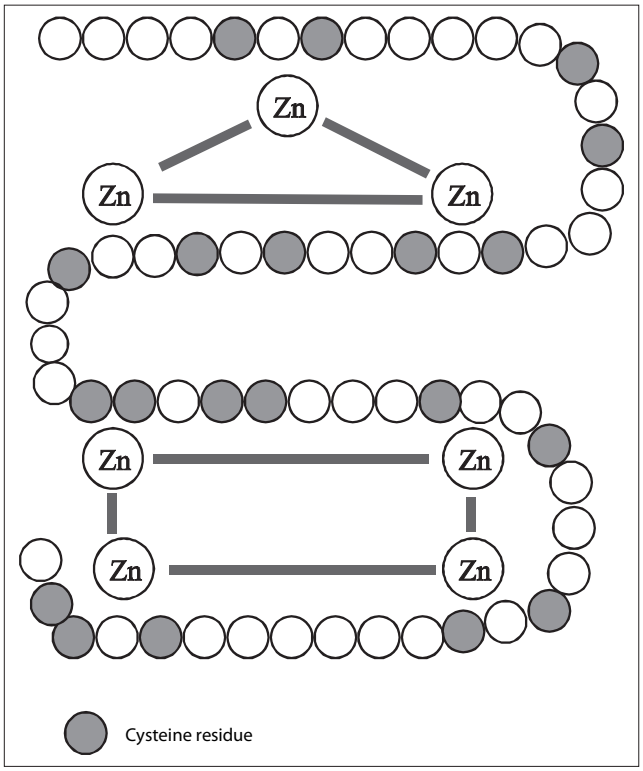

Figure 2. Role of MT in homeostasis of zinc.
Metallothionein molecules contain 20 residues of cysteine, which aproximately amounts to 30% of amino acid content. Large amounts of cysteine with sulfhydril groups determine protein activity. The electrophilic character of sulfur in the sulfhydril groups of the amino acid is responsible for their high affinity to metallic ions. Metallothionein display the highest affinity to metals of the transitory groups (e.g. zinc, cadmium, mercury,copper and silver). One molecule of metallothionein can bind 7 atoms of bivalent metals, e.g. zinc. The role of metallothionein as protein involved in the metabolism of metals indispensable for growth, development and functioning of an organism is now a fact. MT are a reservoir of zinc and copper ions. They provide macromolecules requiring zinc and copper with those microelements

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. MTs are a reservoir of zinc and copper ions (Zn and Cu). They provide macromolecules requiring zinc and copper with those microelements [11].

Zinc in the Nervous System. Zinc (Zn^{2+}) concentration in the mammal brain averages 10 mg/g (wet weight), and remains unchanged in adults. Slight fluctuations in Zn^{2+} are observed in growing organisms, after which the concentration becomes stabilized only to reach constant levels in maturity. Zn^{2+} concentration in the blood serum and in extra-cellular fluid roughly amounts to 0.15mM [12]. Nervous cells, like most eucariotic cells, accumulate zinc, which is required for growth and development [1]. In neuronal cells, zinc concentration may reach the values of 150 mM and only a small percentage of it is free zinc [13,14].

According to Frederickson [15], in the central nervous system (CNS) there are 3 pools of zinc:

- ca 80% of zinc occurs as protein bound zinc: a bound pool or so called 'inactivated' zinc;
- another pool of zinc occurs in the synaptic vesicles – this pool can be exposed through histochemical staining and constitutes about 10% of the overall zinc content in a cell. This zinc locally co-exists with glutaminic acid and, similar to glutaminic acid, is released into the synaptic space [16].
- still another pool of zinc, so called 'free' zinc, is not bound to proteins.

Zinc in the Synaptic Vesicles. Many neurons (zincergic neurons), and some other types of secretory cells (e.g. beta-pancreatic cells), accumulate large amounts of zinc in the secretory vesicles. For example, the synaptic vesicles of glutaminergic neurons in the cerebral cortex and the limbic system contain significant amounts of zinc which are released on excitation. Zinc is the most slowly exchangeable with other cellular compartments and the cytosol. The type of Zinc which occurs in cellular organellae, and the free zinc is otherwise called labile zinc because of its susceptibility to metallic chelators [6].

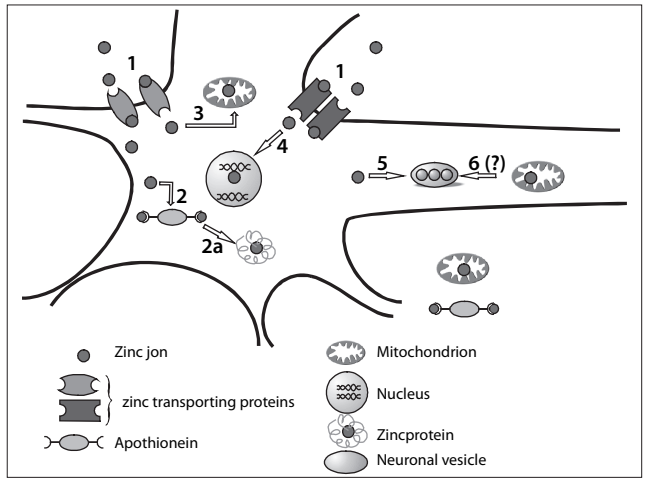

Figure 3. Zinc homeostasis in neurons.
1 – zinc transportation from the cellular environment through cellular membrane using transporting proteins.
2 – in the cytosole, zinc is bound by metallothioneins.
2a – metallothioneins not only bind zinc, but are also the main proteins responsible for its delivery to zinc-requiring proteins.
3 – mitochondria are sub-cellular elements which function as buffers: they store large amounts of Zn when zinc concentration reaches a critical point.
4 – a certain amount of cytosol zinc is utilized in the nucleus: Zn serves, among other things, as a structural element for transcription factors there.
5 – considerabe amount of free zinc is stored in the synaptic vesicles and moved toward the axon terminal – its pre-synaptic part.
6 – a hypothetical path of transferring zinc into the synaptic vesicles through a mitochondria-synaptic vesicle junction.

Zinc is moved toward the axon terminal, its concentration increasing in the synaptic vesicles (Fig. 3.). In the pre-synaptic area, about 20% of overall, free cellular zinc accumulates [17]. It is interesting that mitochondria also prevail in the same area, but it is unclear whether such a location is purposeful, and whether it favours exchange of zinc ions between the mitochondria and the synaptic vesicles. The connections between cellular organellae, which serve the exchange of, e.g. calcium ions, have been described in the literature. They take place after the creation of connections between the mitochondria and the endoplasmatic reticulum, and/or between the mitochondria and the SOK channels in the cellular membrane [18].

On stimulating glutaminergic neurons, zinc is released from the synaptic vesicles into the synaptic space. The level of synaptic zinc then increases to micromolar values [14]. The mechanism of zinc efflux into the synaptic space is unclear. It is known, however, that even at physiological concentrations there is a change in the activity of GABA and glutaminic (NMDA) receptors [19]. Among other things, a synaptic release of Zn^{2+} is required for long-term potentiations of the hippocampal CA3 pyramidal neurons [20-22].

If we assume that zinc is a neuromodulator, then after Zn release from the synaptic vesicles, and after fulfilling its signal-modulator function, there must be some kind of mechanism whereby the synapses rid themselves of zinc, and the synaptic vesicles refill. It seems that ZIP proteins are actively involved in the clearance of the synapses and the refilling of the synaptic vesicles.

Moreover, those proteins probably retrieve zinc from metallothioneins present in the cytoplasm to form synaptic vesicles. Alternatively, zinc may leave the synapse through diffusion, and can be uptaken by glia [21].

Excess of Zinc in the Synapses can be Toxic. Studies confirm that the toxicity of zinc shows up when there is an increase in the third fraction or free zinc in a cell. The increase in Zn^{2+} level can be triggered by some factors that cause damage to the mechanisms maintaining the physiological values of zinc [23-26]. The authors suggest that 300 nM is a toxic value for cortical neurons.

The neurotoxicity of zinc has been demonstrated on animal models in which a stroke, ischemia, Alzheimer's disease, or convulsions were induced [23,27-30]. The detailed mechanism of the toxic activity of zinc is not known, but it seems that the main cause of the neuronal death is low energy production. The question arises about how the mitochondria – the subcell entities specializing in energy production – contribute to the death of a neuron [31-34].

Intracellular concentration of free zinc reaching 400-600 nM causes neuronal death in cell cultures [13,35]. In living organisms, a similar situation can occur during hypoxia or chronic epileptic excitation. Large amounts of zinc and glutamate are then released from the synaptic vesicles and enter the post-synaptic cells.

In such pathological conditions, zinc uptake by the mitochondria increases, which causes structural and functioning disturbances, and creates conditions favourable for the initiation of cell apoptosis and death [32,33,36,37].

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