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Metal-ligand forms of iron and zinc in the human body

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Abstract

Metals have a wide range of effects on biological processes, playing an important role in maintaining the functioning of the human body. However, many metals, including essential elements, can have a toxic effect on the body, leading to pathological processes. The biological role of an element depends on a number of physicochemical facts, such as the oxidation degree and the formation of metal-ligand organic and inorganic complexes. For example, most of the iron binds to transferrin and ferritin ensuring the safe transportation of the fenton-active trivalent metal ions in the bloodstream. Free Fe^{3+} ions lead to the formation of reactive oxygen species and further damage of cell structures. Thus, the chemical form of the element determines the toxicokinetics and toxicodynamics of metals. Knowledge in total exposure of elements in biological fluids is not enough to understand the complex mechanism of biological and abnormal reactions. It is necessary to study the interaction of metal elements with various ligands such as high- and low-molecular compounds (proteins, polysaccharides, nucleic acids, citrates, amino acids). In this regard, the application of modern analytical methods is becoming increasingly important to obtain qualitative and quantitative data on elements, ionic forms, speciation and functions in biological systems. The combination of these methods is called "speciation analysis", which is a well-established way to study the biological role and metabolism of trace elements. This article reviews the main metal-ligand forms of iron (transferrin, albumin, ferritin and citrate) and zinc (albumin, α_2-macroglobulin, IgG, transcuprein, metallothioneins, ZIP and ZnT transporters). This information can be useful both in fundamental and applied researches in the biology and medicine.

Keywords: metallomics, elemental analysis, speciation analysis, iron, zinc.

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Background

Information about the chemical speciation of elements is of immense importance for understanding issues in the field of nutrition, biochemistry, medicine, and pharmacology [1–3]. The development of modern instrumental analytical methods enables the performance of a reliable chemical species analysis, which makes it possible to determine the elementary form and relationships with biological ligands [4].

Speciation analysis represents a type of chemical analysis, the essence of which is determination of the qualitative and quantitative content of various forms of a chemical element present in the test sample [5]. Metal-ligand analysis offers a distinct advantage, since over the past decades, evidence has been obtained that determination of the gross concentration of an element in a biological sample is not sufficient to assess its essentiality or toxicity [6, 7].

This analysis includes a complex of highly sensitive physicochemical analytical methods, which include chromatographic, spectroscopic, ionization, and diffraction methods [8, 9]. Currently, the most attention is paid to so-called hybrid analytical methods, which provide high selectivity [5]. In such methods, as a rule, the preliminary separation of components is combined with their subsequent detection. In this regard, high-performance liquid chromatography followed by mass spectrometry with inductively coupled plasma is most commonly used. The combination of these methods serves as a universal approach to separating the forms of chemical elements with an effective method to determine ultratrace amounts of a wide range of chemical elements [5, 10].

Studies have shown that different compounds of the same element can have varying effects, since the biological functions of metals depend on several characteristics [11]. Thus, the valence state, isotopic form, and attached ligands affect the functional roles of metals. For example, Cr(III) is essential, while Cr(VI) is highly toxic and promotes the

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development of cancer [12–14]. Inorganic forms of Se, for example, selenite and selenate, are considered neurotoxic [15], while the selenoproteins P and glutathione peroxidase, by contrast, are neuroprotective [16, 17]. The absorption capacity of Fe(II) iron is lower than that of Fe (III), but only Fe (II) is effective in correcting Fe deficiency in the body, which is important when creating nutritional supplements [18].

Achievements in the field of analytical chemistry have provided proof that metabolic disorders can occur not only as a result of a deficiency or excess of a certain element but also due to the interaction between various metal ions and the presence of metal-binding (chelating) agents [19, 20]. In studies conducted by Skalny et al. [21], the metal-ligand forms of several elements in the blood serum of patients with Parkinson's disease were significantly altered. A significant decrease in the content of the Cu/ceruloplasmin complex was noted against an increase in the levels of low-molecular-weight forms (amino acids) associated with copper. The level of Mn-albumin complexes in the subjects was more than four times higher than that in the control group [21].

German scientists analyzed the speciation of selenium in the cerebrospinal fluid of patients with Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease [22, 23]. The researchers showed that the level of neurotoxic forms of selenium did not change in patients with Parkinson's disease, in contrast to those with other neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease. There was a significant difference in the ratio of albumin-bound selenium and selenomethionine (Se-HSA/Se-Met) between patients with Parkinson's disease and those with amyotrophic lateral sclerosis [15]. In the future, similar studies may help to establish new diagnostic biomarkers with potential utility in clinical practice.

It should be noted that the chemical nature of zinc and iron ligands is not completely known, and there is also insufficient data on changes in the levels of metal-ligand forms of these elements in various diseases. In this regard, the following review compiles the current information on possible metal-ligand forms of these metals and their functions.

Metallomics of iron

Iron (Fe) is a vital trace element that serves as a cofactor of hemoproteins and nonheme-containing proteins, including many enzymes [24]. Hemoproteins are involved in numerous biological reactions, such as oxygen binding and transport (hemoglobin), oxygen metabolism (catalases, peroxidases), cellular respiration, and electron transport (cytochromes). Proteins containing nonheme iron are important for cellular processes such as deoxyribonucleic acid (DNA) synthesis and cell proliferation and differentiation [25].

Impairment of iron homeostasis is associated with various diseases. For example, iron deficiency resulting from poor absorption or distribution of the metal causes anemia. Excess iron leads to its deposition in tissues and becomes the pathophysiological basis of numerous diseases, including cancer and a number of neurodegenerative conditions [26, 27]. When iron levels are high, free iron(II) is formed, which can cause oxidative stress and cell death through lipid peroxidation, known as ferroptosis [28].

Oxidative stress is closely related to the balance of the Fe(II)/Fe(III) redox pair. While Fe(III) is oxidatively inactive, Fe(II) promotes the formation of reactive oxygen species by catalyzing the decomposition of H_2O_2 and subsequent formation of hydroxyl radicals and peroxidation of membrane lipids [29]. In this regard, scientists note that quantitative measurements of iron, including the analysis of metal-ligand and ionic forms, rather than its gross determination, are keys to a deeper understanding of pathological processes [30, 31].

The human body contains approximately 3–5 g of iron [32], the majority in the hemoglobin of erythroid cells (>2 g) or muscle myoglobin (~300 mg) in the form of heme. Macrophages in the spleen, liver, and bone marrow contain a temporary fraction of iron (~600 mg), while excess of the metal is stored in the liver parenchyma as ferritin (~1000 mg). All other cellular iron-containing proteins and enzymes bind a total of approximately 8 mg of iron [33].

The most common types of iron are Fe (II) and Fe (III). The redox potential of iron can vary depending on the attached ligands, for example, ferryl (Fe^{4+}) can be temporarily generated as an intermediate product in metal-mediated oxidative transformations [25]. Some of the metal-ligand forms of iron are transferrin, albumin, ferritin, and citrate.

1. *Transferrin*. In the human body, iron metabolism is a strictly regulated process, where iron enters the bloodstream from the intestine in the form of a divalent Fe(II) cation, which is easily oxidized to the state of Fe(III) and bound by transferrin (80 kDa) [34]. Plasma transferrin is the most important physiological source of iron synthesized in the liver. Together with ferritin, it binds almost all of the iron circulating in the plasma. Under physiological conditions, this chelation maintains a low level of free iron in the bloodstream to prevent the

formation of reactive oxygen species and promotes the transport of iron into cells [35].

Iron-bound transferrin distributes iron to other cells in the body by binding to receptors on cell surfaces, which is followed by iron import into the cell by means of endocytosis. Once in the cytoplasm, iron is delivered to various intracellular sites, including mitochondria for heme biosynthesis, and to ferritin, which serves as an intracellular iron depot [36]. Metabolically inactive iron is stored in ferritin and is in equilibrium with exchangeable iron bound to carrier molecules [37].

2. *Ferritin*. Ferritin is a 450 kDa complex that binds iron oxyhydroxide particles (up to 4500 iron atoms). Most ferritins are located inside cells and serve to store iron. The physiological role of blood-borne extracellular ferritins is less clearly defined [38].

3. *Albumin*. Human serum albumin, known as a low-affinity iron-binding protein, has been proposed as a ligand for the pool of nontransferrin-bound iron, which is normally present in patients with iron excess [39]. Albumin carries a net negative charge with a large amount of carboxylic acids on the molecule surface, due to which potential iron-binding sites can form [40, 41].

4. *Citrate*. Earlier studies of the chemical speciation of elements in human blood plasma assumed the presence of only the trivalent form of iron in it and indicated that among the low-molecular-weight ligands, iron is present exclusively in the form of a hydroxycitrate complex [42]. However, it has now been established that blood plasma and serum contain two to six types of low-molecular-weight iron complexes. Citrate, acetate, pyruvate, and phosphates are potential low-molecular-weight metal ligands. Considering the affinity of each ligand, scientists have suggested that citrate is the dominant ligand of nontransferrin-bound iron [43].

Thus, assessment of the levels of iron complexes with high- and low-molecular-weight ligands *in vivo* is key to a better understanding of the metabolism of this trace element, as well as the various pathologies associated with impaired iron metabolism.

Metallomics of zinc

Zinc (Zn) is the second most common and indispensable trace element in a living organism. Researchers have identified more than 3,000 zinc proteins that are essential for enzymatic and structural functions; transport and storage; and the repair, replication, and translation of DNA [44, 45]. Six classes of enzymes (oxidoreductases, transferases, hydrolases, lyses, isomerases, and ligases) use zinc as a cofactor [46, 47]. During enzymatic processes, zinc plays catalytic, coactive (increasing or decreasing catalytic functions), and structural roles (the latter is necessary for the stability of the quaternary structure of enzymes) [48, 49]. Scientists suggest that more than 10% of the human genome encodes zinc proteins [50].

Impairment of zinc homeostasis can cause many chronic diseases, such as neurological disorders, autoimmune and age-related degenerative diseases, diabetes mellitus, atherosclerosis, and a number of malignant neoplasms. It can enhance oxidative stress and lead to the formation of inflammatory cytokines [51–56].

Currently, zinc deficiency is prevalent, especially in developing countries. According to a World Health Organization report, approximately 2 billion people worldwide have zinc deficiency, which ranks fifth among causes of death and morbidity. In industrialized countries, zinc deficiency is most common in the elderly population [57].

Due to mechanisms regulating the concentration of zinc in the body at the sites of zinc absorption with food (small intestine) and endogenous excretion (intestine and kidneys), its toxicity is rare. However, zinc loading can interfere with copper absorption and cause copper deficiency [58].

Zinc is present in all tissues of the body, but its highest concentrations are present in skeletal muscles (60%), bones (30%), liver, and skin (5%), with the remaining 2%–3% found in other tissues and organs (such as the brain, kidneys, and pancreas) [59]. Following absorption by cells, zinc is distributed in the cytoplasm (50%), nucleus (up to 40%), and cell membrane (10%) [60].

Unlike iron and copper, zinc is a redox-neutral element and has only one valence state, Zn(II) [61]. This is because its filled d-orbital excludes participation in redox reactions [62]. For this reason, zinc is of key importance as a structural, catalytic, and signal component.

It is reported that 75%–90% of total plasma zinc binds to serum albumin, and this fraction constitutes the majority of the plasma zinc exchange pool [63]. Approximately 10% of plasma zinc binds tightly to α_2-macroglobulin. Less than 1% of total plasma zinc forms low-molecular-weight complexes with amino acids (histidine and cysteine) [64, 65]. Serum zinc accounts for approximately 0.1%; 80% of it binds freely to albumin and 20% to α_2-macroglobulin [66].

Absorption and excretion in the gastrointestinal tract are effective mechanisms for maintaining zinc homeostasis [67]. According to published studies, zinc absorption occurs at the highest rate in the jejunum. Excessive endogenous zinc is excreted from the body with the feces [68, 69].

Thus, during zinc deficiency or limited dietary intake, zinc excretion decreases, while intestinal absorption increases. Conversely, in the presence of excess zinc, excretion increases, while absorption is not affected. As a result, zinc levels in tissues and blood plasma remain stable [70].

Intracellular zinc homeostasis is highly regulated. Because Zn cannot freely cross cell membranes, there are several transmembrane ion carriers. Proteins are the main ligands of zinc(II) ions. There are many zinc-binding proteins, such as albumin; α_2-macroglobulin; haptoglobulin; classes G, M, and A immunoglobulins (Ig); complement fraction C4; prealbumin; and C-reactive protein [71]. Zinc-binding proteins can act as zinc storage compounds to maintain immunoregulatory and oxidative balance. The coordination environment of zinc in proteins is limited by oxygen, nitrogen, and sulfur donors from the side chains of amino acids (histidine, glutamate, aspartate, and cysteine) [61].

According to the analysis of literature data, the following zinc complexes exist.

1. *Albumin*. Serum albumin is a single-chain protein of approximately 66 kDa, which is the main protein component of blood plasma, responsible for the circulatory transport of a number of molecules (fatty acids, hormones, metal ions, and drugs) [63]. Albumin has several metal-binding sites that are specific to various ions. The authors note that the serum level of fatty acids affects metal binding. Zinc-binding capacity may be reduced when fatty acids are bound to albumin [72]. Studies performed on isolated, perfused rat intestines have shown that albumin is responsible for the transport of Zn^{2+} to the liver [73]. It was revealed that albumin promotes the incorporation of Zn^{2+} ions into endothelial cells and erythrocytes [74].

2. *α_2-Macroglobulin* is a protein with a high affinity for zinc. Zinc ions are necessary for protein activation and binding of α_2-macroglobulin to cytokines [75]. Studies of human blood plasma show that two identical α_2-macroglobulin subunits with a molecular weight of approximately 182 kDa associate via disulfide bonding to form a tetrameric structure [76].

3. *IgG binding.* According to Yamanaka et al. [68], IgG can specifically bind zinc ions through the Fc domain. The data obtained demonstrate that the γ-globulin molecule contains several zinc-binding sites [77, 78]. Thus, when interacting with zinc ions distributed in the periglobular space, metal complexes are formed that acquire new effector functions compared to those exhibited by γ-globulins in the native region [79].

4. *Transcupreine* is a high-affinity copper carrier in blood plasma (mass: 250 kDa), participating in the initial distribution of copper entering the blood from the digestive tract. However, scientists at the University of California indicate that zinc can bind to this protein [80].

There are other zinc-binding proteins that control zinc homeostasis.

5. *ZIP transporters*. Proteins of the ZIP transporter family transfer zinc into the cytosol from the extracellular space and intracellular compartments [81]. According to published research, the human genome encodes 14 types of ZIP transporters. Their genes are designated *SLC39A1–SLC39A14*, and they code for ZIP1–ZIP14 proteins, respectively. These transporters are expressed in various tissues and cells (such as the brain, liver, pancreas, and kidneys), and their proteins are localized to various subcellular compartments (such as the plasma membrane, lysosomes, and mitochondria) [82].

6. *Zinc transporters (ZnTs).* Another group is represented by zinc transporters (ZnTs), which transport zinc from the cytosol to the extracellular space and intracellular organelles [83]. Scientists have identified nine ZnTs, designated ZnT1–ZnT8 and ZnT10, encoded by the *SLC30A1–SLC30A8* and *SLC30A10* genes, respectively [84, 85]. Both families of transporters respond to zinc deficiency and excess by enacting specific changes in their subcellular localization and protein stability. Dysregulation and mutations in transporter genes can cause functional disorders [86].

Thus, ZIP transporters increase the level of cytoplasmic zinc, while ZnTs decrease it [60, 87].

7. *Metallothioneins*. In addition to these carriers, there are metallothioneins, which are low-molecular-weight metal-binding proteins (6–7 kDa) with a high level of cysteine, which are able to bind cellular zinc through thiol clusters (up to seven zinc atoms) [88, 89]. Four classes of metallothioneins are known. MT-1 and MT-2 are ubiquitous in the body, maintain cellular homeostasis of zinc and copper, and chelate heavy metals (cadmium, mercury) in order to reduce cytotoxicity and their intracellular concentrations. MT-3 and MT-4 are localized mainly in the brain and stratified epithelial tissues [90]. The highest concentrations of metallothionein are found in the liver, kidneys, intestines, and pancreas [91].

8. *Proteins of the S100 family* can bind zinc ions. The binding of transition metals by S100 proteins was first characterized more than 30 years ago [92]. The functions of these proteins are predominantly regulatory in nature; they are involved in several processes, including proliferation, differentiation, and inflammation [93]. Most S100 proteins have a common homodimeric structure, in which approximately 10 kDa monomers are assembled to form a compact α-helix [94].

According to a literature analysis, Zn-binding proteins include S100A1, S100A2, S100A3, S100A4, S100A5, S100A6, S100A7, S100A8/9, S100A12, S100A15, S100A16, and S100B [95]. The zinc-binding site in proteins S100B, S100A6, S100A7, S100A8/A9, S100A12, and S100A15 consists of three histidine residues and one aspartate residue, or four histidine residues, whereas S100A2, S100A3, and S100A4 have cysteine-containing zinc-binding sites [94]. The role of such binding has not been fully studied; however, it is assumed that divalent cations, including not only calcium but also zinc, copper, and manganese, affect the oligomerization of S100 proteins and, consequently, their functional ability [96, 97].

9. *Zinc complexes with anions*. Hydrogen sulfide, hydrogen phosphate, and sulfate are the strongest inorganic zinc-binding anions. Diphosphate ($P_2O_7^{4-}$), triphosphate ($P_3O_{10}^{5-}$), and tetraphosphate ($P_4O_{13}^{6-}$), as well as inositol phosphate, bind zinc much more efficiently. Acetate (CH_3COO^-), hydrocarbonate (HCO_3^-), and chloride (Cl^-) are ligands with an intermediate Zn^{2+} coordination strength. For example, hydrocarbonate is a ligand for the zinc enzyme carbonic anhydrase, and chloride has been identified as a zinc ligand in the crystal structures of some zinc proteins [98]. Organic acids (pyruvate, succinate, glutarate, lactate, folate, oxaloacetate, and citrate) can also be potential zinc ligands [61]. In the cell, all these anions are also buffered; there is a controlled balance between the free and bound forms.

10. *Other complexing compounds*. Glutathione (GSH), adenosine triphosphate (ATP), citrate, and amino acids are low-molecular-weight zinc ligands [99]. Glutathione serves to detoxify xenobiotics and heavy metals, restore protein thiols, maintain cell membranes, and deactivate free radicals. Its oxidized dimer (GSSG) controls the content of metals in metallothionein [100]. Zinc has been reported to form complexes with glutathione: Zn(GSH) and $Zn(GSH)_2$ [101]. Moreover, the formation of a ternary complex, Zn (II)-GSH-His, has been observed [100].

ATP has been revealed to serve as a zinc ligand. Thus, some kinases prefer the ZnATP complex to the MgATP complex [99], for example, a bound ZnATP complex was found in the crystal structures of flavokinase and pyridoxalkinase [102].

$Zn(His)^+$ has been established as the preferred substrate for membrane transport. Histidine forms 1:1 and 2:1 complexes with zinc, where the 2:1 $Zn(His)_2$ complex has no overall charge [103]. The Zn(II) ion, which is essential for the stability and structure of the zinc finger, is tetrahedrally bound by cysteine thiol groups [101].

Another mechanism regulating the intracellular zinc concentration is its accumulation in vesicles in the form of chelated and labile Zn. For example, approximately 20% of zinc is located in the synaptic vesicles of glutamatergic neurons in the hippocampus and cerebral cortex [104, 105]. In addition to the zinc-containing granules and vesicles found under normal physiological conditions, zinc also accumulates in subcellular compartments under certain pathological conditions. When zinc concentrations are high, cytosolic vesicles called zincosomes appear [106]. However, at present, these formations are poorly studied, and there are no data on the chemical speciation of zinc in them. Thus, the study of zinc complex formation is of great importance for biochemistry, since various forms of this microelement are involved in diverse biological processes.

Conclusion

The development of analytical chemistry methods has led to the understanding that the total concentration of chemical elements cannot provide complete information about their bioavailability and possible toxic effects on ecological systems and living organisms. Only knowledge of the chemical form of an element can provide information about possible chemical and biochemical processes and thus lead to a greater understanding of the toxicity or essentiality of the element. For this reason, the determination of the chemical form of elements is of great practical importance.

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