ASSESSING THE POSSIBILITY OF INTERACTIONS OF VARIOUS METALS WITH ALPHA-2-MACROGLOBULIN AND OTHER HUMAN BLOOD PROTEINS *IN VITRO*

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Homeostasis of metals plays an important role in functioning of the body. Not only the concentrations of toxic and essential metals in bodily fluids, but also their ability of interaction with proteins and enzymes defining the enzyme activity, are important. The study was aimed to compare the possibilities of binding interactions between various metal ions and human serum proteins. Chemical reactions between the immobilized metal ions (Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Hg^{+}, Cd^{2+}, Pb^{2+}, Cr^{3+}, Co^{2+}, Ag^{+}, Bi^{2+}, Ba^{2+}, Sr^{2+}) and the serum proteins or highly purified blood metalloprotein (alpha-2-macroglobulin, α2M) were assessed by the crossed immunoelectrophoresis with *in situ* adsorption in the second dimension. It has been shown that Hg^{+}, Cu^{2+}, Zn^{2+}, Cd^{2+} ions more actively interact with metalloproteins (particularly, with α2M) and many other human blood proteins in *in vitro* reactions than other ions. We have demonstrated that α2M interacts not only with Zn^{2+} and Cd^{2+} ions, as earlier reported, but also with Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, Sr^{2}, Ag^{+}. Interaction of a number of metal ions, including highly toxic ones, with blood proteins that are not metalloproteins has been revealed. The findings confirm the fundamental possibility of the metal ion imbalance active involvement in metabolic disorders via effects on the body's regulatory and transport proteins, which requires further investigation

Keywords: metal ions, metalloproteins, alpha-2-macroglobulin, immunoelectrophoresis, intoxication

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Compliance with ethical standards: the study was carried out in accordance with the principles stated in the Declaration of Helsinki of the World Medical Association.

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ИЗУЧЕНИЕ ВОЗМОЖНОСТИ ВЗАИМОДЕЙСТВИЯ РАЗЛИЧНЫХ МЕТАЛЛОВ С АЛЬФА-2-МАКРОГЛОБУЛИНОМ И ДРУГИМИ БЕЛКАМИ КРОВИ ЧЕЛОВЕКА *IN VITRO*

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Гомеостаз металлов играет важную роль в жизнедеятельности организма. При этом имеет значение не только концентрация токсичных и эссенциальных металлов в биологических жидкостях, но и их способность взаимодействовать с белками и ферментами, определяющая активность последних. Целью работы было сравнить возможности связывания различных ионов металлов с белками сыворотки крови человека. Изучение реакций иммобилизованных ионов металлов (Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, Fe^{3+}, Mg^{2+}, Hg^{+}, Cd^{2+}, Pb^{2+}, Cr^{3+}, Co^{2+}, Ag^{+}, Bi^{2+}, Ba^{2+}, Sr^{2+}) с белками крови, а также с высокоочищенным металлопротеином крови (альфа-2-макроглобулин, а2-МГ) проводили методом перекрестного иммуноэлектрофореза с адсорбцией *in situ* во втором направлении. Показано, что в реакциях *in vitro* ионы Hg^{+}, Cu^{2+}, Zn^{2+}, Cd^{2+} активнее других взаимодействуют с металлопротеинами (в частности с а2-МГ) и со многими другими белками крови человека. Продемонстрировано, что а2-МГ взаимодействует не только с ионами Zn^{2+} и Cd^{2+}, как описано ранее, но и с Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}, Pb^{2+}, Sr^{2}, Ag^{+}. Выявлено взаимодействие ряда ионов металлов, в том числе высокотоксичных, с белками крови, не являющимися металлопротеинами. Результаты подтверждают принципиальную возможность активного участия дисбаланса ионов металлов в обменных нарушениях через воздействие на регуляторные и транспортные белки организма, что требует дальнейшего изучения.

Ключевые слова: ионы металлов, металлопротеины, альфа-2-макроглобулин, иммуноэлектрофорез, интоксикация

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Соблюдение этических стандартов: исследование проведено в соответствии с принципами Хельсинкской декларации Всемирной медицинской ассоциации.

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Metal ions play an important role in metabolic pathways of living systems, from electron transfer and biocatalytic reactions to shaping the tertiary structure of metalloproteins that determines their biological activity. The disturbed essential metal homeostasis is associated with functional impairment and severe disorders. It is well-known that the non-physiological concentrations of Fe, Mn, Cu, Zn cause manifestations of neurotoxicity, while the excess levels of Zn and Cu trigger the toxicity-induced damage to the kidney, liver, cardiovascular system, gastrointestinal tract, and inhibit the function of the immune and central nervous systems [1]. Substantial amounts of metals are found in plaques, Lewy bodies, and cytoplasmic inclusions of the cells of individuals with neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, etc.) and amyotrophic lateral sclerosis [2]. Furthermore, Zn ions provide antiatherogenic properties [3]. Deficiency of essential

metals also has an adverse effect on the body. In particular, Zn ions are essential for realization of the enzyme (alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase and superoxide dismutase) function, and the Zn deficiency that triggers dermatoses, anorexia, and growth retardation, is observed in individuals with slow wound healing and impaired reproductive function [1]. Metals with variable valence may exert both positive and negative effects: Mn ions promote generation of the hydroxyl radical and at the same time are involved in the development of atherosclerosis as cofactors of antioxidant enzymes [3].

When assessing their effects on the body, it is extremely important to take into account reactions of metals, including metals that form part of organic compounds, enzymes and proteins, with each other. In particular, highly toxic Pb, Hg, Cd cause severe intoxication when ingested [4], and organoselenium compounds demonstrate antioxidant and antitoxic activity in individual poisoned with salts of heavy metals [5]. In general, about one third of serum proteins contain ions of metals [2]. Of greatest interest is the study of metalloproteins performing regulatory and transport functions that are capable of exerting indirect effects on the number of organs and systems of the body due to changes in ionic composition. In particular, human alpha-2-macroglobulin (α2M) contains four Zn ions. The levels of this protein are rather high (2–3 g/L), and its functions are diverse: inhibition of the broad spectrum of proteases; transportation and regulation of the synthesis of cytokines, hormones and growth factors; regulation of apoptosis, synaptogenesis, neuron growth, and proliferation; regulation of dopamine concentrations in dopaminergic neurons and synthesis of choline acetyl transferase [6].

When studying the role of metal ions in physiological processes, it is important to consider the distribution of those in tissues and bodily fluids. In particular, Ag, Ca, Cu, In, Li, Na, Se, Si, Sr ions are found mostly in human blood plasma, while Fe, K, Mn, Ni, V, Zn are found in blood cells. The distribution of heavy metals shifts from cell to blood plasma with the increase in ionic raduis, and vice versa the distribution of alkali metals shifts to cells [7]. Therefore, it is advantageous to use the inductively coupled plasma mass spectrometry (ICP-MS) allowing one to identify a broad spectrum of metals, including minor concentrations, to assess the whole body levels of metals. However, other methods should be used to study interactions with proteins: crystallography, nuclear magnetic resonance, electron paramagnetic resonance, fluorescence-based methods, spectrometry and surface plasmon resonance [2]. The vast majority of the above methods involve partial or complete protein denaturation and can hardly be used for analysis of mixtures. This makes it more difficult to obtain objective scientific data on the processes that take place in the living organism. The less sensitive but more gentle methods with good potential of comparative visualization of the results, such as variants of crossed immunoelectrophoresis, seem to be promising.

The study was aimed to compare the possibilities of binding interactions between various metal ions and human serum proteins.

METHODS

The study involved drainage blood serum obtained from 40 generally healthy donors of both genders aged 20–40 in order to identify all possible interactions.

The highly purified alpha-2-macroglobulin (α2M) preparations were obtained from blood plasma by using the combination of fractional precipitation with PEG 6000, anion-exchange chromatography, and zinc-chelate chromatography [8].

Polyclonal rabbit antisera against all human blood proteins and human α2M were obtained by intradermal immunization of two groups of rabbits (with blood serum and highly purified α2M preparation, respectively).

The possibility of binding interactions between human serum proteins and metal ions was assessed using the crossed immunoelectrophoresis with *in situ* adsorption in the second dimension. Immunoelectrophoresis was run in the horizontal agarose gel slabs on the glass plates. For that type 1 agarose (Sigma; USA) solution in the 1% Tris Tricine buffer (pH 8.6) was used [9]. The round wells were cut out in the 1 mm thick gel layer formed, to which 5 μL of blood serum or α2M preparation per well were introduced. Electrophoresis in the first dimension was run for 1 h at 200 V. Then gel was cut into 10 mm wide strips that were moved to the edges of glass plates. Free space was poured with 1% agarose sol.

When the gel was formed, we cut out a 0.5 cm wide pouch approximately 2 mm from the border with the gel used for electrophoresis in the first dimension. The pouch freed from agarose was filled with gel with essential, conditionally essential, and toxic metals the salts consisted of, immobilized onto sorbent (Table 1).

To make gel, the sample of iminodiacetic acid agarose (IDA) (Sigma; USA) was placed on a chromatography column and washed successively with ten volumes of the following preparations: 1) 0.05 M ethylenediaminetetraacetic acid disodium salt; 2) double distilled water; 3) 0.05 M aqueous solution of the metal to be tested; 4) double distilled water; 5) Tris Tricine buffer, pH 8.6.

After filling the pouch with gel with immobilized metal, the agarose gel above the pouch was cut at 2 mm away from the pouch edge, and the vacant space was filled with 1% agarose sol containing 5% of appropriate antiserum. Immunoelectrophoresis in the second dimension was run for 18 h at 100 V. At the end of electrophoresis the gel plates were washed for 24 h with the 0.1 M NaCl solution, dried, and stained with the Coomassie Brilliant Blue (R-250) dye.

RESULTS

According to the data obtained by studying the samples of drainage blood serum collected from healthy donors (Fig. 1), blood proteins bound to both essential and toxic metals contained in intermediate gel with appropriate decrease in the precipitate area in an electropherogram.

In particular, active binding of many serum proteins was observed in the presence of not only Zn^{2+},Cu^{2+} and Cd^{2+} ions, but also Hg^{+} in intermediate gel.

Moderate binding to serum proteins was found in the electropherograms, in which intermediate gel contained not only Sr^{2+} and Pb^{2+}, but also Ba^{2+}. Moreover, active binding of Sr^{2+} to serum glycoproteins was observed.

Weak affinity of certain serum proteins not only to Fe^{3+} and Mn^{2+} ions, but also to Ag^{+} was reported. Weak binding interactions between certain proteins and Ca^{2+} and Bi^{2+} ions was shown.

Low binding of Mg^{2+} ions to γ-globulins was revealed. Weak interactions between certain proteins and Co^{2+} ions were observed. Assessment of interactions with Cr^{3+} ions showed almost no reactions.

Assessment of the possibility of binding interactions between the highly purified α2M molecules and the metal ions (Fig. 2) showed that this metalloprotein which was also a

Table 1. List of substances used for immobilization of metals

№	Sample	№	Sample	№	Sample	№	Sample
1	control (no metals)	2	$CuSO_4$	3	$CdSO_4 \times 3\ H_2O$	4	$AgNO_3$
5	$Zn(CH_3COO)_2 \times 2\ H_2O$	6	$FeCl_3 \times 6\ H_2O$	7	$Pb(CH_3COO)_2$	8	$Bi(NO_3) \times 5\ H_2O$
9	$MnCl_2 \times 4\ H_2O$	10	$CoCl_2 \times 6\ H_2O$	11	$Sr(NO_3)_2$	12	$Ba(CH_3COO)_2$
13	$CrCl_3$	14	$CaCl_2 \times 6\ H_2O$	15	$MgCl_2 \times 6\ H_2O$	16	$Hg(NO_3) \times 1/2\ H_2O$

glycoprotein actively interacted not only with Zn^{2+} contained in intermediate gel, but also with Cd^{2+} ions (peak height reduction by more than 50% of the baseline was reported). When the intermediate gel contained not only Fe^{3+}, Mn^{2+}, Ca^{2+}, Mg^{2+} ions, but also Pb^{2+}, $Sr2^{+}$, and Ag^{+}, the peak height was 50–60% of the peak height observed in the electropherogram of the reference sample.

When the immobilized Cu^{2+}, Hg^{+}, Ba^{2+} ions were included, the peak height reduction observed in the electropherogram was 40% or more, which was indicative of rather active interaction between these metals and the α2M molecule *in vitro*.

Inclusion of Cr^{3+} and Co^{2+} ions in intermediate gel resulted in the lowest binding to α2M, and the peak height reduction did not exceed 10% compared to the reference sample.

Semi-quantitative data on the intensity of the serum proteins' and α2M binding to the metal ions contained in intermediate gel are provided in Table 2.

DISCUSSION

The study has shown that serum proteins are capable of binding to immobilized metals, even if they are not metalloproteins.

The fundamental possibility of protein binding without forming the metal chelate bonds is well-known, however, only binding to essential metals was earlier identified by using the combination of gel filtration chromatography and the inductively coupled plasma atomic emission spectroscopy (ICP-AES): two proteins interacted with zinc (alpha-2-macroglobulin and albumin), two proteins bound to iron (ferritin and transferrin), and four proteins bound to copper (ceruloplasmin, albumin, factor V, transcuprein) [10]. According to the results obtained by other authors, the use of the combination of affinity chromatography (immobilized metal contained in the sorbent) and LC-MS-MS showed that complement component 3 (C3), α2M, certain albumin isoforms, apolipoproteins, ceruloplasmin, serotransferrin, keratin, γ-globulins could interact not only with essential Cu^{2+}, Zn^{2+}, but also with the conditionally essential Cd^{2+}, Pb^{2+} [1, 11].

According to our findings, the spectrum of blood proteins capable of interacting with metal ions is even broader, it includes interactions with macroelements (Ca^{2+}, Mg^{2+}) and toxic microelements (Ag^{+}, Hg^{+}, Ba^{2+}, Bi^{2+}).

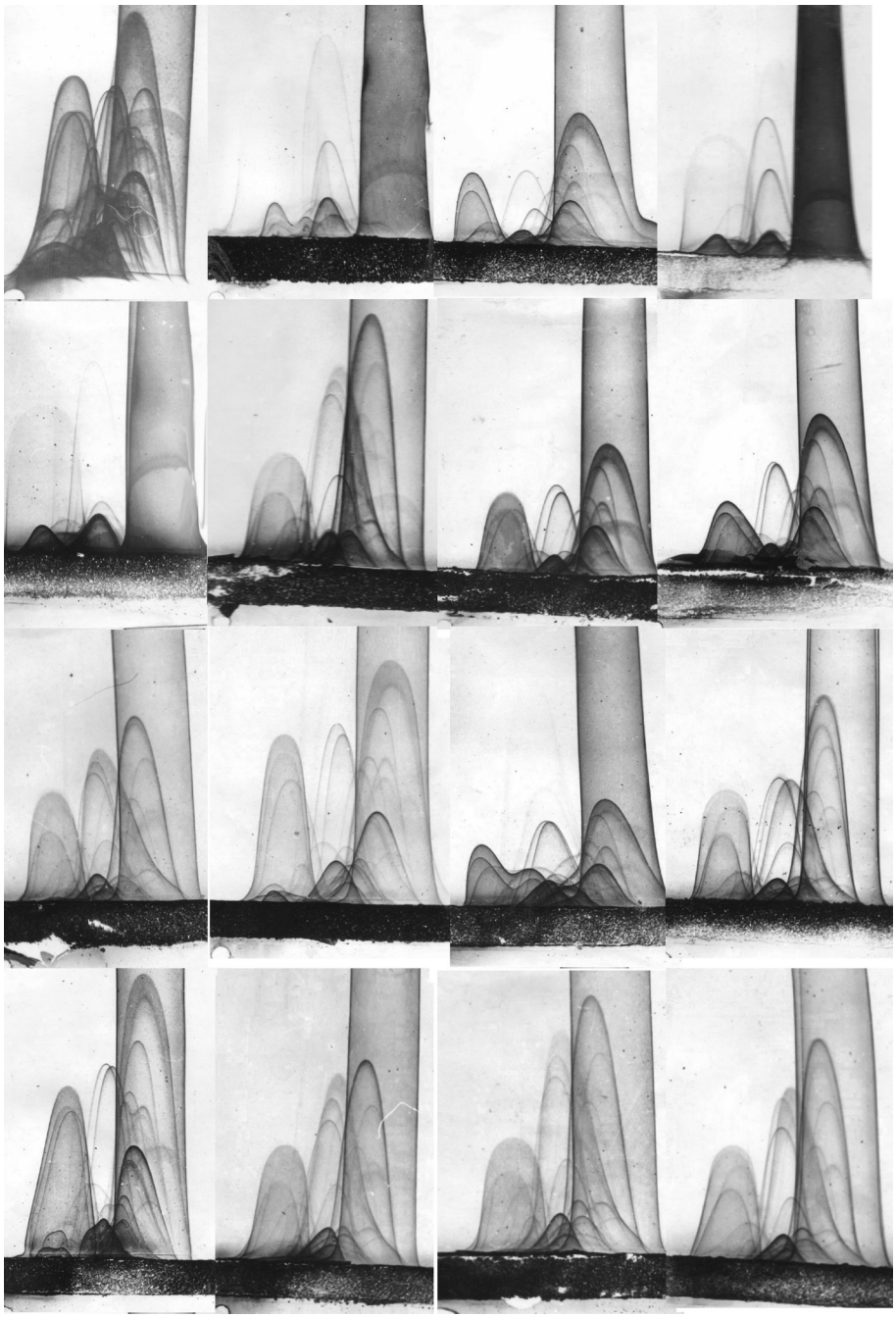
Certainly, the results of *in vitro* study need to be further confirmed by *in vivo* study, however, it can be assumed that metal toxicity may be also realized through competitive interactions with essential metals contained in proteins, as well as through formation of the metal–metal bonds that are not very strong but are capable of negatively affecting the protein conformation structure and its affinity to receptors and ligands, as is the case with competitive replacement of essential microelement. The underlying patterns and biological effects of such reactions in acute and chronic metal toxicity require further investigation.

According to the findings, it is not only the ions of essential microelements (Cu^{2+}, Zn^{2+}) that show high affinity (at the known level of assumptions, considering the identified interactions and the currently known properties) to the wide variety of serum proteins, as has been previously reported after studying the protein fraction of human γ-globulins and opposite effects of copper and zinc [11] together with Cd^{2+} attributable to conditionally essential metals, but also the Hg^{+} ions. It is well-known that many cadmium compounds are toxic. The thiol groups (–SH) of cysteines that are found in proteins are the most important targets for Cd^{2+}: cadmium can suppress the activity of many mitochondrial enzymes [12]. It can be assumed that when there are competitive or other interactions between cadmium ions and regulatory or transport metalloproteins or enzymes containing Zn^{2+} and especially the less reactive Fe^{3+} Cu^{2+}, Mg^{2+} ions, these can significantly change properties of proteins, inhibit their original functions and even show new properties (such as immunogenicity). For good reason, among other things, administration of albumin, also a zinc-containing metalloprotein, is recommended when treating cadmium poisoning.

Mercury toxicity is well known. Among other things, mercury competes for metal (zinc, copper and other) binding

Table 2. Binding affinity of human serum proteins to the studied metal ions contained in intermediate gel during immunoelectrophoresis

Object	Binding affinity	Ions of macroelements and essential microelements	Ions of conditionally essential microelements	Ions of toxic microelements
All serum proteins	+++ (high)	Cu^{2+}, Zn^{2+}	Cd^{2+}	Hg^{+}
	++ (moderate)		Pb^{2+}, Sr^{2+}	Ba^{2+}
	+ (weak)	Ca^{2+}, Mn^{2+}, Fe^{3+}		Ag^{+}, Bi^{2+}
	+/– (low)	Mg^{2+}, Cr^{3+}, Co^{2+}		
Alpha-2-macroglobulin	+++ (≥ 50%)	Zn^{2+}	Cd^{2+}	
	++ (≥ 40%)	Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+}	Pb^{2+}, Sr^{2+}	Ag^{+},
	+ (≥ 30%)	Cu^{2+}		Hg^{+}, Ba^{2+}, Bi^{2+}
	+/– (< 10%)	Cr^{3+}, Co^{2+}		



Control	Cu^{2+}	Cd^{2+}	Ag^{+}
Zn^{2+}	Fe^{3+}	Pb^{2+}	Bi^{2+}
Mn^{2+}	Co^{2+}	Sr^{2+}	Ba^{2+}
Cr^{3+}	Ca^{2+}	Mg^{2+}	Hg^{+}

Fig. 1. Interaction of immobilized metal ions with serum proteins. The reduced height and area of the peak are indicative of interaction between protein and metal contained in intermediate gel. The layout of metals in the samples is in table

sites in metalloproteins, thereby suppressing their activity, and demonstrates active high affinity interactions with the thiol, carboxylic, and other enzymes (ATPase, cholinesterase, alkaline phosphatase, glutathione peroxidase, glutamine synthetase, etc.) [5, 13]. Our findings show that mercury ions also can interact with other proteins. Biological effects of such interactions require further investigation.

The rather high reactivity of barium and lead ions relative to various human serum proteins also attracts attention. Despite the fact that lead is considered to be a conditionally essential microelement, most of lead compounds (especially the water-soluble ones) are toxic. It is well-known that the mechanism underlying toxic effects of lead is associated with inhibition of the thiol enzymes, interaction with the carboxyl and phosphate groups of biopolymers, and inactivation of esterases [14]. Our findings have clearly demonstrated the presence of such interactions with human blood proteins.

Despite the fact that copper is an essential microelement, and copper excess and deprivation have the extremely adverse effects on the body, pathogenesis of various neurodegenerative

diseases is associated with disturbed copper homeostasis [4]. It has been previously shown that Cu^{2+} possesses properties of the redox-active metal that realizes its high oxidation potential in biological systems. The Cu^{2+} chelation can cause partial breakdown of the side amino acid radicals, sugars, and sialic acids on the surface of macromolecule, as well as destructuring of the surface layer spatial arrangement, including the antigen determinants [11]. The identified broad spectrum of proteins that interact with copper ions substantiates the need for further identification aimed at clarifying their possible role in pathogenesis of various disorders.

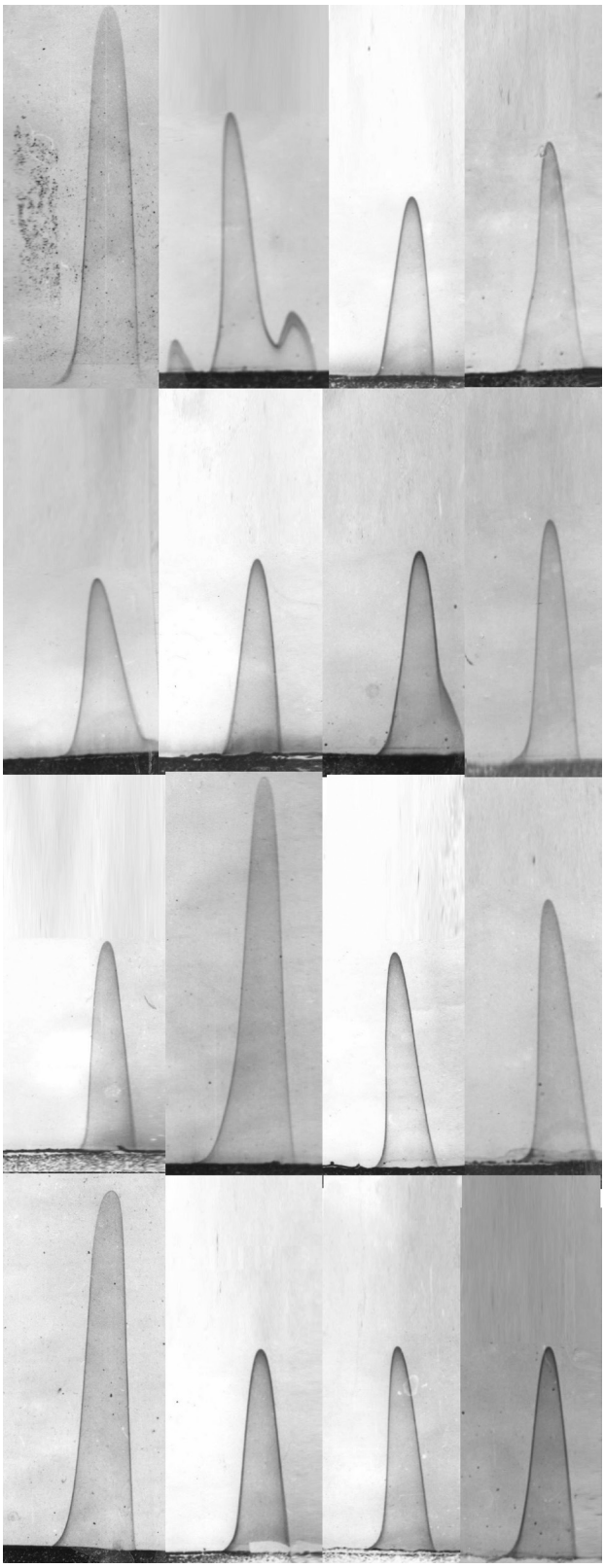
α2M was selected as a "model" protein for comparative assessment of the possibility of binding to metal ions. The study involved the use of protein in its native state obtained by gentle preparative low-pressure chromatography methods. This brought the results obtained closer to real processes taking place in the human body.

The observed interactions between α2M and Zn^{2+} and Cd^{2+} ions were the most intense. This result matches those on the α2M, Cd^{2+}, Zn^{2+} bonding and to a lesser extent with those on the α2M, Ni^{2+}, and Pb^{2+} bonding obtained earlier by other methods [1]. It is well-known that zinc is a structure-forming component of α2M, each of four α2M subunits contains one zinc ion. It has been previously shown that cadmium, that interacts with zinc contained in α2M, breaks the chelating bond between half-molecules, and the protein that is split into two parts loses most of its regulatory functions. Since α2M is involved in regulation of the cytokine profile, lipid metabolism, inhibition of the broad spectrum of proteases, signal transduction in the nervous system, inflammatory and autoimmune responses of the body [6], cadmium toxicity may result in massive failure of regulatory processes involving this protein, and the presence of such interaction, in turn, explains some mechanisms underlying cadmium toxicity.

Furthermore, zinc metabolism disorders play a key role in aging. Some authors recommend to use zinc as a dietary supplement to increase life expectancy [15]. It is obvious that cadmium that competes for binding to α2M has the exact opposite effect. In contrast to zinc ions contained in proteins, excess concentrations of free zinc in blood exert neurotoxic effects [4]. This can be one of the components of accelerated aging associated with chronic metal toxicity and one of the components of the senile dementia pathogenesis.

The identified interaction between α2M and lead is also important. It has been previously shown that lead can interact with active centers of a number of enzymes (ATPase, glucose-6-phosphate dehydrogenase, alkaline phosphatase, etc.) [16]. It is known that α2M is a universal proteinase inhibitor, however, in this case the direct effects of Pb^{2+} on this enzyme inhibitor are observed instead of indirect ones. It can be assumed that circulation of the lead complexes with α2M along with the lead phosphates and albuminates takes place in lead toxicity [14]. Considering the lead hepatotoxicity, adverse effects on α2M come from two sources: death of the α2M-producing hepatocytes and inhibition of the α2M function via interaction with lead ions.

The identified ability of α2M to interact with manganese ions also can adversely affect the functions of this regulatory and transport protein. It is known that excess manganese disrupts catalytic activity of enzymes, and the reduced form (Mn^{3+}) contributes to oxidative stress [17]. In this case the adverse effects of this essential microelement on α2M can be realized via two mechanisms: change in functions of protein itself due to competing interaction involving zinc replacement and damage to the α2M molecule caused by superoxide radicals. It is



Control	Cu^{2+}	Cd^{2+}	Ag^{+}
Zn^{2+}	Fe^{3+}	Pb^{2+}	Bi^{2+}
Mn^{2+}	Co^{2+}	Sr^{2+}	Ba^{2+}
Cr^{3+}	Ca^{2+}	Mg^{2+}	Hg^{+}

Fig. 2. Interaction of immobilized metal ions with alpha-2-macroglobulin from blood serum. The reduced height and area of the peak are indicative of interaction between protein and metal contained in intermediate gel. The layout of metals in the samples is in table

known that the "oxidized" form of α2M shows impaired ability of utilization (reduced receptor affinity) [6] and becomes potentially immunogenic due to altered conformation.

The previously described increased inhibition of the activated protein C (APC) in blood that involves α2M and occurs when exposed to ions of divalent metals (Zn, Mn, Cu) is indirect evidence of the influence of microelement imbalance on the physiological and pathological processes involving regulatory and transport proteins [18]. According to the findings, this phenomenon may be caused by the direct effects of essential microelements on α2M and its functions in the body.

The identified α2M ability of active interaction with macroelements (magnesium, calcium, iron) requires further investigation of the impact of such bonding on physiological and pathological processes.

The fact that most of clinical manifestations of acute metal toxicity (albeit less severe) are found in many conditionally healthy residents of large industrial cities attracts attention. These manifestations include conduction disorders and impaired limb sensitivity, frequent headaches and chronic fatigue, increase in the number of individuals with cognitive impairment and signs of early onset dementia, impaired liver function, etc. The findings suggest that the identified interactions of toxic and essential metals with the regulatory and transport proteins may affect the development of functional disorders and pathological processes.

CONCLUSIONS

Most of human serum proteins, including those that are not metalloproteins, interact with metal ions in the *in vitro* experiment. High intensity of protein interaction with the conditionally essential cadmium and toxic mercury suggests that pathogenetic mechanisms of intoxication with these metals may be realized via blood protein structural and functional impairment. The identified *in vitro* α2M metalloprotein interaction with the conditionally essential and toxic metals may take the form of the competing metal–metal interactions and adversely affect the structure and functions of this regulatory and transport protein *in vivo*. The mechanisms underlying interaction and reversibility of protein binding to metals *in vivo* require further investigation.

References

1. Wang F, Chmil C, Pierce F, Ganapathy K, Gump B, MacKenzie J, Mechref Y, Bendinskas K. Immobilized metal affinity chromatography and human serum proteomics. Journal of Chromatography B. 2013; 934: 26–33.
2. Witkowska D, Rowińska-Żyrek M, Witkowska D, Rowińska-Żyrek M. Biophysical approaches for the study of metal-protein interactions. Journal of Inorganic Biochemistry. 2019; 199: 110783.
3. Lozhkin AP, Biktagirov TB, Abdulyanov VA, Gorshkov OV, Timonina EV, Mamin GV, i dr. Marganec v aterogeneze: obnaruzhenie, proisxozhdenie i rol'. Biomedicinskaya ximiya. 2012; 58 (3): 291–99. Russian.
4. Aschner M, Costa LG. Neurotoxicity of Metals. Springer International Publishing AG, 2017; 383 p.
5. Ruseckaya NYu, Borodulin VB. Biologicheskaya aktivnost' selenorganicheskix soedinenij pri intoksikacii solyami tyazhelyx metallov. Biomedicinskaya ximiya. 2015; 61 (4): 449–61. Russian.
6. Zorina VN, Zorina RM, Zorin NA. Osobennosti vzaimodejstviya belkov semejstva makroglobulinov mezhdu soboj i s receptorami ehndocitoza (vozmozhnyj mexanizm transmembrannogo perenosa). Biomedicinskaya ximiya. 2011; 57 (1): 106–13. Russian.
7. Barashkov GK, Zajceva LI, Kondaxchan MA, Konstantinova EA, Raspredelenie ximicheskix ehlementov v cel'noj krovi i plazme. Biomedicinskaya ximiya. 2003; 49 (3): 297–302. Russian.
8. Zorin NA, Zorina RM, Zorina VN. Poluchenie preparatov α-makroglobulina s zadannymi svojstvami. Gematologiya i transfuziologiya. 2000; 45 (5): 20–21. Russian.
9. Emmet M, Crowle AJ. Crossed immunoelectrophoresis: qualitative and quantitative considerations. J Immunol Meth. 1982; 50: R65–R83.
10. Manley SA, Byrns S, Lyon AW, Brown P, Gailer J. Simultaneous Cu-, Fe-, and Zn- specific detection of metalloproteins contained in rabbit plasma by size-exclusion chromatography–inductively coupled plasma atomic emission spectroscopy. Biol Inorg Chem. 2009; 14: 61–74.
11. Cheknyov SB. Belki γ-globulinovoj frakcii, xelatiruyushhie kationy metallov, v fiziologicheskoj immunoregulyacii. Oppozitnye ehffekty medi i cinka. Immunologiya. 2021; 42 (3): 293–300. Russian.
12. Genchi G, Sinicropi M, Lauria G, Carocci A, Catalano A. The Effects of Cadmium Toxicity. Int J Environ Res Public Health. 2020; 17 (11): 3782.
13. Ynalvez R, Gutierrez J, Gonzalez-Cantu H. Mini-review: toxicity of mercury as a consequence of enzyme alteration. Biometals. 2016; 29 (5): 781–8.
14. Koshkina VS. Kotlyar NN, Kotelnikova LV, Dolgushina NA. Kliniko-toksikologicheskaya xarakteristika svinca i ego soedinenij. Medicinskie novosti. 2013; 1: 20–25. Russian.
15. Mocchegiani E, Costarelli L, Giacconi R, Cipriano C, Muti E, Malavolta M. Zinc-binding proteins (metallothionein and a-2 macroglobulin) and immunosenescence. Experimental Gerontology. 2006; 41: 1094–7.
16. Assi MA, Hezmee MN, Haron AW, Sabri MY, Rajion MA. The detrimental effects of lead on human and animal health. Vet World. 2016; 9 (6): 660–71.
17. Mazunina DL. Negativnye ehffekty marganca pri xronicheskom postuplenii v organizm s pit'evoj vodoj. Ehkologiya cheloveka. 2015; 3: 25–31. Russian.
18. Heeb MJ, Gruber A, Griffin JH. Identification of Divalent Metal Ion-dependent Inhibition of Activated Protein Cbycr2-Macroglobulin and cr2-Antiplasminin Blood and Comparisons to Inhibition of Factor Xa, Thrombin, and Plasmin. The Jounal of Biological chemistry. 1991; 266 (26): 17606–12.

Литература

1. Wang F, Chmil C, Pierce F, Ganapathy K, Gump B, MacKenzie J, Mechref Y, Bendinskas K. Immobilized metal affinity chromatography and human serum proteomics. Journal of Chromatography B. 2013; 934: 26–33.
2. Witkowska D, Rowińska-Żyrek M, Witkowska D, Rowińska-Żyrek M. Biophysical approaches for the study of metal-protein interactions. Journal of Inorganic Biochemistry. 2019; 199: 110783.
3. Ложкин А. П., Биктагиров Т. Б., Абдульянов В. А., Горшков О. В., Тимонина Е. В., Мамин Г. В., и др. Марганец в атерогенезе: обнаружение, происхождение и роль. Биомедицинская химия. 2012; 58 (3): 291–99.
4. Aschner M, Costa LG. Neurotoxicity of Metals. Springer International Publishing AG, 2017; 383 p.
5. Русецкая Н. Ю., Бородулин В. Б. Биологическая активность селенорганических соединений при интоксикации солями

тяжелых металлов. Биомедицинская химия. 2015; 61 (4): 449–61.
6. Зорина В. Н., Зорина Р. М., Зорин Н. А. Особенности взаимодействия белков семейства макроглобулинов между собой и с рецепторами эндоцитоза (возможный механизм трансмембранного переноса). Биомедицинская химия. 2011; 57 (1): 106–13.
7. Барашков Г. К., Зайцева Л. И., Кондахчан М. А., Константинова Е. А., Распределение химических элементов в цельной крови и плазме. Биомедицинская химия. 2003; 49 (3): 297–302.
8. Зорин Н. А., Зорина Р. М., Зорина В. Н. Получение препаратов α-макроглобулина с заданными свойствами. Гематология и трансфузиология. 2000; 45 (5): 20–21.
9. Emmet M, Crowle AJ. Crossed immunoelectrophoresis: qualitative and quantitative considerations. J Immunol Meth. 1982; 50: R65–R83.
10. Manley SA, Byrns S, Lyon AW, Brown P, Gailer J. Simultaneous Cu-, Fe-, and Zn- specific detection of metalloproteins contained in rabbit plasma by size-exclusion chromatography–inductively coupled plasma atomic emission spectroscopy. Biol Inorg Chem. 2009; 14: 61–74.
11. Чекнёв С. Б. Белки γ-глобулиновой фракции, хелатирующие катионы металлов, в физиологической иммунорегуляции. Оппозитные эффекты меди и цинка. Иммунология. 2021; 42 (3): 293–300.
12. Genchi G, Sinicropi M, Lauria G, Carocci A, Catalano A. The Effects of Cadmium Toxicity. Int J Environ Res Public Health. 2020; 17 (11): 3782.
13. Ynalvez R, Gutierrez J, Gonzalez-Cantu H. Mini-review: toxicity of mercury as a consequence of enzyme alteration. Biometals. 2016; 29 (5): 781–8.
14. Кошкина В. С., Котляр Н. Н., Котельникова Л. В., Долгушина Н. А. Клинико-токсикологическая характеристика свинца и его соединений. Медицинские новости. 2013; 1: 20–25.
15. Mocchegiani E, Costarelli L, Giacconi R, Cipriano C, Muti E, Malavolta M. Zinc-binding proteins (metallothionein and a-2 macroglobulin) and immunosenescence. Experimental Gerontology. 2006; 41: 1094–7.
16. Assi MA, Hezmee MN, Haron AW, Sabri MY, Rajion MA. The detrimental effects of lead on human and animal health. Vet World. 2016; 9 (6): 660–71.
17. Мазунина Д. Л. Негативные эффекты марганца при хроническом поступлении в организм с питьевой водой. Экология человека. 2015; 3: 25–31.
18. Heeb MJ, Gruber A, Griffin JH. Identification of Divalent Metal Ion-dependent Inhibition of Activated Protein Cbycr2-Macroglobulin and cr2-Antiplasminin Blood and Comparisons to Inhibition of Factor Xa, Thrombin, and Plasmin. The Jounal of Biological chemistry. 1991; 266 (26): 17606–12.