Reactions between Human Serum γ-Globulin and Zinc Cations

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Interactions of human serum γ-globulin with zinc cations in solution were studied by differential spectrophotometry in UV light. Supraphysiological concentrations of zinc caused an increment in optical density of protein solution reflecting the effect of γ-globulin saturation with the metal. Zinc concentrations below physiological led to hypochromism in the protein absorption spectrum. Conformation changes in γ-globulin during interactions with zinc are analyzed for the surface and intramolecular binding sites and are compared with the effects of copper cations.

Key Words: *γ-globulin; zinc cations; interaction*

Our previous studies validated the concept on the capacity of γ-globulin proteins to carry copper cations. This concept is based on the data on γ-globulin interactions with copper by the extra- and intraglobular sites and on the possibility of forming surface and internal protein-metal complexes [3,4]. Biphasic nature of the reaction indicates different time of cation saturation of external and internal coordinating sites differing by the parameters of reactions with copper (presumably, by dissociation constant) and retaining certain stability (at least, in local environment) [4].

The effects of zinc (in contrast to those of copper, which is not regarded as immunoactive trace element) in immune reactions are well studied. The role of zinc in immunogenesis can be different: it determines biological activity of thymulin [7,8], regulates early stages of T-lymphocyte maturation [14], apoptosis [13, 14], plays an important role in the provision of cytokine production and reception [7,14], directly participates in the main immune reactions [8,14].

Physiological content of zinc in the plasma is 10-20 μM [6,10,11], while the concentration of free bioactive cations is 0.2-1.0 nM [6,10]. A variety of zinc-mediated effects suggest that this concentration of free metal is appreciably below its real content and participation in metabolic processes [12]. However, chelating of even negligible amount of cations during their reaction with serum proteins creates appreciable deficiency of zinc in the microenvironment and significantly modifies the immune reactions depending on the metal transport and metabolism [2].

We evaluated conformation changes in γ-globulin and the concentration relationships characterizing protein interaction with zinc cations. The methodological approaches used in this study correspond to our previous experiments with copper [3,4], which makes possible a comparison of the effects of cations with similar and different properties in the realization of biological activity [1,6,9].

MATERIALS AND METHODS

Human serum γ-globulin preparation (Serva) in 0.15 M NaCl (pH 7.14-7.20) at protein concentrations of 50, 100, 150, and 200 μg/ml was used. Large protein associations were removed by filtering through 0.45-μ membrane filters (Millipore) and the samples were incubated for 1 h at 37°C with ZnCl (Zn concentration 0.1-12.0 μg/ml). The reaction was carried out in conical graduated polystyrene 10-ml tubes (Costar); the volu-

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me of each sample was 5 ml. γ-Globulin samples incubated with zinc sulfate and samples containing no zinc ions served as the controls. The same concentrations of protein and cations as in the experiments with ZnCl were used in control tests.

The reaction was evaluated by UV spectrophotometry (λ=190-320 nm) with a 10-nm step in the semiautomated mode on a PU 8730 UV/VIS spectrophotometer (Phillips). Changes in the optical density and molar ratios in the solution were estimated on the basis of γ-globulin concentrations determined by absorption at λ=280 nm (extinction coefficient 0.7). pH of the solution was controlled by Expert-001 electronic pH-meter/ionometer (Econics-Expert).

RESULTS

The presence of zinc cations in the solution changes γ-globulin absorption spectrum. The effects of zinc in supraphysiological concentrations (1-12 μg/ml) manifested in increased optical density of the protein solution. Absorption increased for the entire γ-globulin spectrum, the maximum increment being observed at λ=250-270 nm (this range included phenylalanine chromophore absorption band) and was recorded in 280-290 nm zone corresponding to absorbtion of tyrosine and tryptophane residues.

Reactions of γ-globulin with zinc cations, evaluated spectrally at λ=260 and λ=280 nm (similarly as in experiments with copper) indicated saturation of protein with the metal (Fig. 1). The pattern of the detected relationship confirms that zinc in concentrations surpassing the physiological reacts with ligands on the surface of protein molecule. This reduces the compactness of the globule packing and induces its unfolding into extramolecular space. Simultaneously some carbohydrates and amino acids located in the interdomain region of γ-globulin are released into the water phase; these components hidden in the native protein molecule can now mediate intermolecular hydrophobic and other interactions. This is associated with the formation of supramolecular protein aggregations in γ-globulin solution, which manifests in opalescence or turbidity increasing in parallel with the increase in zinc cation concentration.

Spectral changes in γ-globulin solution are less pronounced than in experiments with copper: the increment in optical density in the presence of saturating concentration of zinc is recorded at a 2.0-2.5 times lower level, while the saturating concentration 6 μg/ml is several times higher than that for copper [4]. In contrast to the effects of copper cations, zinc causes no precipitation of γ-globulin agglomerates.

The increment in optical density of γ-globulin solution reacting with zinc in supraphysiological concentrations is characterized by exponential dependence on protein content in the sample (Fig. 2). The results attest to cooperative nature of the studied effect. Binding of zinc cations by some amino acid residues creates conformation prerequisites for exposure of numerous sites capable of reacting with the metal. Simultaneous increase in γ-globulin content in the sample sharply increases the number of zinc-binding sites; occupation of these binding sites seems to trigger changes in the molecule characterized by cooperativity during interaction with the metal. During this interaction 150 μg/ml protein binds 1.0 μg/ml zinc (*i.e.* one γ-globulin molecule binds 30 cations) (Fig. 2).

The detected relationship corresponds to that observed in experiments with copper [4]. The effect of zinc was appreciably lower than that of copper: even at the level of the maximum increment in the optical density it reaches only half of the spectral effect induced by the presence of copper [4].

Zinc in concentrations of 0.1-0.5 μg/ml interacting with γ-globulin induces spectrum hypochromism, most pronounced in the spectrum band corresponding to absorption of aromatic amino acid chromophores. In the differential UV spectra this area is located at λ=250-270 nm with a peak at λ=260 nm. In distraction UV spectra it is pronounced at λ=220 nm and reaches the maximum at zinc concentration of 0.5 μg/ml. The detected hypochromism, similarly as in experiments with copper [3,4], attests to increased compactness of the protein molecule in the presence of low zinc concentrations, which seems to result from metal incorporation into the internal compartments of the protein globule.

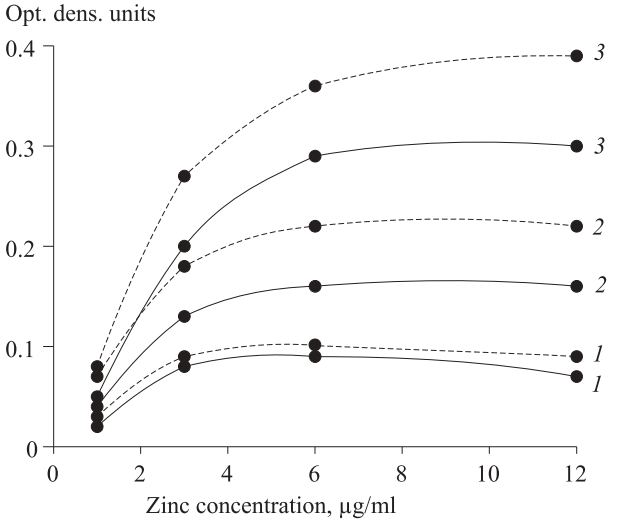

Fig. 1. Increment in the optical density of human serum γ-globulin solution in the presence of zinc in supraphysiological concentrations. Here and in Fig. 3: continuous lines: λ=280 nm; interrupted lines: λ=260 nm. γ-Globulin concentrations: *1*) 50 μg/ml; *2*) 100 μg/ml; *3*) 150 μg/ml.

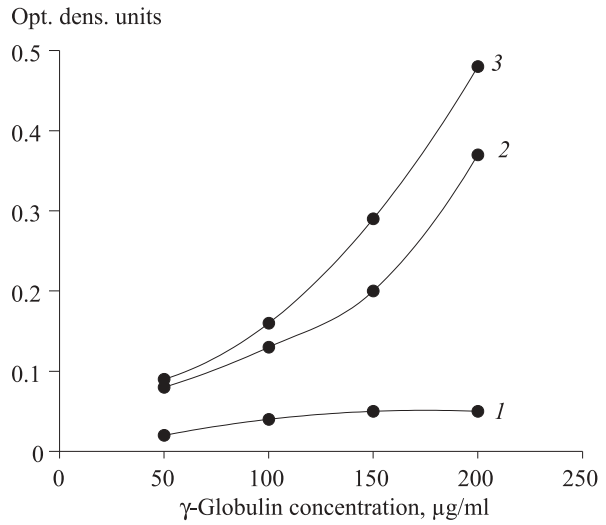

Fig. 2. Relationship between the increment in optical density of γ-globulin solution during reaction with zinc cations and protein concentration in the sample (λ=280 nm). Zinc concentration: *1*) 1 μg/ml; *2*) 3 μg/ml; *3*) 6 μg/ml.

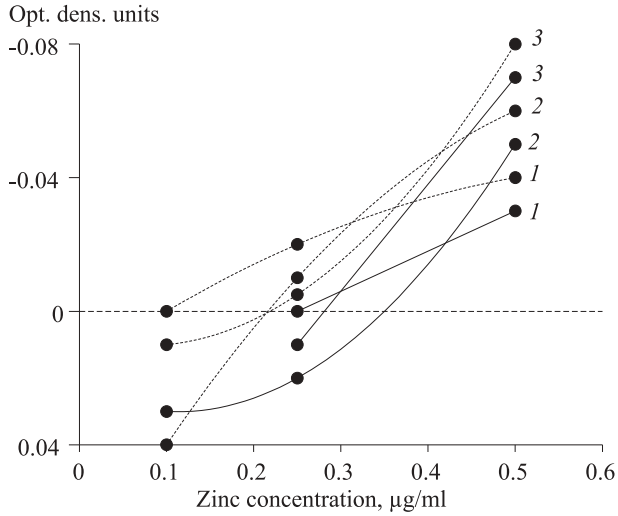

Fig. 3. Manifestations of γ-globulin absorption spectrum hypochromism in the presence of low concentrations of Zn.

Under certain conditions (at a certain γ-globulin/metal molar ratio) spectrum hypochromism in the presence of zinc, similarly as the increment in optical density in the presence of supraphysiological concentrations of cations, indicates protein saturation with the metal (Fig. 3). In contrast to experiments with copper, zinc interacts with γ-globulin inducing exponential reduction of the optical density of protein solution (Fig. 3). This suggests that incorporation of zinc into the internal compartments of the protein molecule induces the formation of additional metal binding sites.

Hence, copper more actively forms complexes on the surface of protein globule, while zinc is more effective in intraglobular coordination links. The effect of copper can be explained by availability of surface ligands for its binding and by nonspecificity of interaction, while zinc acts more selectively, for example, due to its high affinity for sulfur-containing ligands and capacity to stabilize the amino acid residues with sulfhydryl groups [1].

Biological activity of zinc is a specific feature of these cations in comparison with other bivalent metals. The effects of zinc are opposite to those of iron and copper (inhibition of lymphocyte proliferation). Regulation of T cell functions by zinc and Zn saturation of mechanisms regulating cellular activity at its concentrations of about 10^{-12} M remain unclear [7]. Zinc is an obligate and selective component in the formation of superantigens [9]. It selects IgG differing by the type of light chains under conditions of their binding to plasma glycoproteins [10] and more actively than other bivalent cations regulates intermolecular interactions [11], determines biological functions of thymulin [7,8], acts as a factor stabilizing cell membranes [15] and cytoskeleton [1].

Extremely low concentrations of free zinc in the plasma are due to its active binding with albumin, α_2-macroglobulin, transferrin, calcium-binding proteins [1,14], amino acids (primarily histidine and cystein [1,5]) and amino acid-rich glycoproteins [10]. Albumin [1,10,12], histidine [1,5], and glycoproteins [6,10] are the main carriers of metal ions in the body providing intense cation exchange between circulating and membrane biomolecules. This can be explained by the dimension of the constant of zinc-protein binding, which does not provide stable complexes [9]. Metallothioneins binding up to 7 zinc atoms are considered as zinc donors for stabilization of biomembranes and thymulin conversion into the active form [7,15].

Local concentration of zinc can increase appreciably (30-60 times) under conditions of stimulation and subsequent degranulation of platelets [6,10]. In this case γ-globulin proteins due to their higher rigidity of molecular structure (in comparison with albumin, for instance) and capacity to react with zinc through surface and intramolecular binding sites become important components of cation interception system.

Involvement of γ-globulins into zinc transport and exchange can be responsible for acquisition of new effector properties by these molecules. Realization of these properties largely depends on saturation of metal binding sites of the biomacromolecule and on conformation of these biomacromolecules under certain conditions [2].

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