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Binding of Zinc Cations to Human Serum γ-Globulin

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Binding of zinc cations to human serum γ-globulin was studied by molecular ultrafiltration. The content of free metal in the filtrate was evaluated by reaction with o-phenanthroline. Conformation characteristics of the protein were determined by UV spectrophotometry. Our findings suggest that γ-globulin molecule contains several zinc binding sites differing by corresponding constants and successively occupied with increase in the content of bound metal. The parameters of zinc binding correspond to those obtained in experiments with copper. Conformation status of protein with bound zinc differs significantly from that of protein with bound copper cations.

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

Zinc cations in the composition of metalloenzymes form cross-links in their active centers and are involved in the formation of common structure of the active center and its conformation dynamics [2]. Zinc actively binds to proteins, glycoproteins, and plasma amino acids, which determines a stable level of this cation in biological fluids and tissues [7,11]. The formation of zinc-protein complexes can lead to the appearance and realization of principally new properties of biopolymers [4].

Methods for correction of immunopathological states actively introduced in modern clinical pharmacology suggest therapeutic use of zinc or zinc-containing preparations [8,11,13] and chelation of metal cations maintaining the immunoregulatory balance [9] or inactivating zinc in active centers of enzymes, *e.g.* enzymes playing the key role in tumor tissue remodeling [3].

We previously showed that γ-globulins interact with zinc cations in solution [5,6]. Obvious changes in protein conformation manifesting, depending on zinc concentration, in unfolding of the molecule into extramolecular space or compactization of the globule suggest metal binding by sites located on the surface of γ-globulin molecule or in its interdomain space [5,6].

Specific features of zinc interactions with γ-globulin, detected in comparison with the effects of copper cation, are expected to manifest during the metal binding to protein. We mean copper activity towards external γ-globulin bonds, while zinc seems to be affine for the inner structures of the protein globule [5,6].

MATERIALS AND METHODS

Preparation of human serum γ-globulin (Serva) in 0.15 M NaCl (pH 7.12-7.19) with protein concentration of 100 μg/ml was used. γ-Globulin samples filtered from large associations through 0.45-μ membrane filters (Millipore) were incubated for 1 h at 37°C with clarified $ZnCl_2$ (0.22 μ, Millipore); metal concentrations was 1.0 to 6.0 μg/ml. γ-Globulin samples incubated under the same conditions without $ZnCl_2$ served as the control.

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After incubation experimental and control samples (5.0 ml) were subjected to molecular ultrafiltration on CF-25 cones (Amicon) for 10-15 min at 300*g* and 20°C. The volume in the cones was brought to the initial volume with 0.15 M NaCl solution. Filtration was repeated according to the same protocol. Supernatants were removed from the cones, brought to the initial volumes, and analyzed by UV spectrophotometry in 190-320 nm range with 10-nm step on a PU 8730 UV/VIS spectrophotometer (Phillips) in a semiautomated mode. Spectrophotometric control of the samples was carried out at all previous steps of the study.

The content of free zinc in the filtrate was evaluated by spectrophotometry (λ=226 nm) of complex formation with o-phenanthroline in 0.15 M NaCl at neutral pH. The reaction was performed using a methodological approach developed at our laboratory: o-phenanthroline dilution was selected experimentally for plotting the calibration curve and recording the results, so that the level of its absorption provided a linear relationship between optical density of the resultant complex with zinc and its concentration in the studied range. The concentrations of o-phenanthroline varied from 3.5×10^{-5} to 7.0×10^{-5} M.

Changes in optical density and molar ratios in the solution were calculated from γ-globulin concentration determined spectrophotometrically (λ=280 nm, extinction coefficient 0.7). Acidity of the samples was controlled using an electron pH-meter/ionometer Expert-001 (Econics-Expert).

RESULTS

Incubation of γ-globulin in solution with supraphysiological content of zinc cations led to unfolding of the protein molecule into the periglobular space, which can be regarded as a manifestation of a trend to denaturation of the initial sample [5,6]. Removal of free zinc (not bound to protein) from the solution restored γ-globulin absorption spectrum to a state close to the control and caused a hypochromic effect attesting to compaction of the globule after zinc incorporation into its inner compartments (Fig. 1). Hence, after removal of metal excess γ-globulin with bound zinc cations acquired a conformation more close to native, this indicating its capacity to renaturing. These data are in line with low redox activity of zinc in intermolecular interactions and indicate, similarly as in experiments with copper, that extramolecular unfolding of the protein reacting with metal is reversible.

Zinc concentrations binding to γ-globulin do not exceed 30-40 μM. They surpass physiological concentrations and [7,10] and threshold plasma content of zinc during its therapeutic use [8], but are not toxic for human cells [13]. For this reason γ-globulin with bound zinc is not toxic for cells reacting with it or fixing it on membranes under conditions of metal exchange.

The relationship between optical density increase in the solution of γ-globulin with bound zinc and the amount of bound metal indicates successive filling of protein sites of different spatial location with cations. Binding starts from the inner regions of γ-globulin molecule and causes its pronounced compaction (Fig. 2). Metal binding to external sites of the globule leads to its unfolding into the extramolecular space with simultaneous increase of absorption in γ-globulin solution. This takes place before binding of the amount of zinc (about 24 μM) causing expression of previously hidden metal-binding sites. Further binding of cations is again realized in the inner sites of the molecule, the cations filling the inner regions of the protein globule or bonds opened as a result of conformation changes in the molecule during fixation of zinc to outer bonds. Optical density of the solution starts to decrease because of compaction, natural under these conditions (Fig. 2). The inner compartments of the molecule are saturated after binding of about 40 μM zinc (Fig. 2). Then the metal fixation continues at the outer bonds of γ-globulin, causing a second unfolding of the structure containing zinc inside the globule.

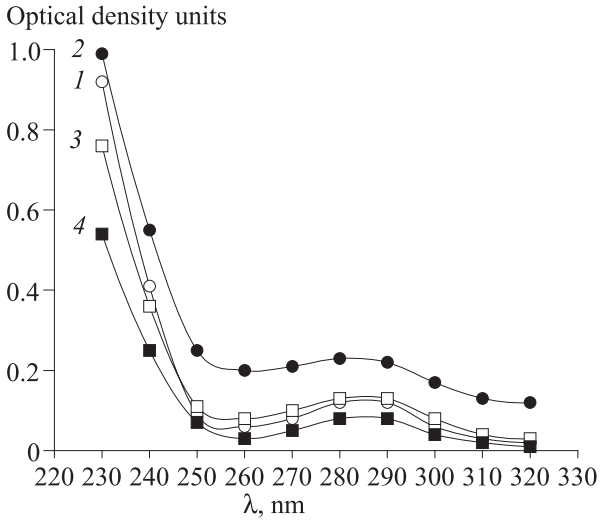

Fig. 1. UV absorption spectra of human serum γ-globulin during binding of zinc cations. Protein spectra: *1*) after 1-h incubation at 37°C in 0.15 M NaCl solution; *2*) after 1-h incubation at 37°C in the same solution containing $ZnCl_2$ with 5.0 μg/ml zinc; *3*) restored in 0.15 M NaCl solution after control (without zinc) ultrafiltration; *4*) with bound zinc and restored in 0.15 M NaCl after removal of free metal by molecular ultrafiltration.

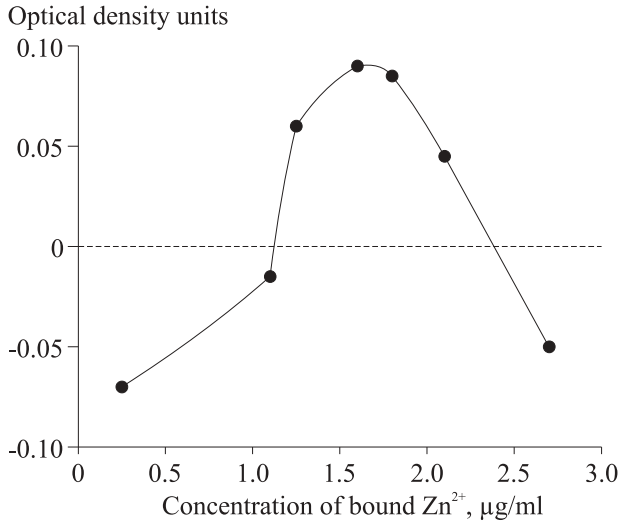

Fig. 2. Changes in absorption parameters of solution of human serum γ-globulin with bound zinc cations. Evaluation at λ=280 nm.

These data are confirmed by Scatchard's curves (Fig. 3). Similarly as in experiments with copper [1], these curves helped to detect three groups of metal-binding sites in the protein globule; these sites are characterized by different, though close, binding constants (Fig. 3). The projections of segments on the abscissa do not overlap. Hence, the metal is bound at sites of different spatial location, occupied by cations stage-by-stage (with saturation of certain sites of the protein molecule with zinc). Abrupt "jumps" from one group of sites to another indicate that zinc binding centers in γ-globulin are not equivalent and mutually dependent (Fig. 3).

Jump-like conformation transitions are characteristic of copper-binding (but not zinc-binding) centers in biomacromolecules. Their presence under

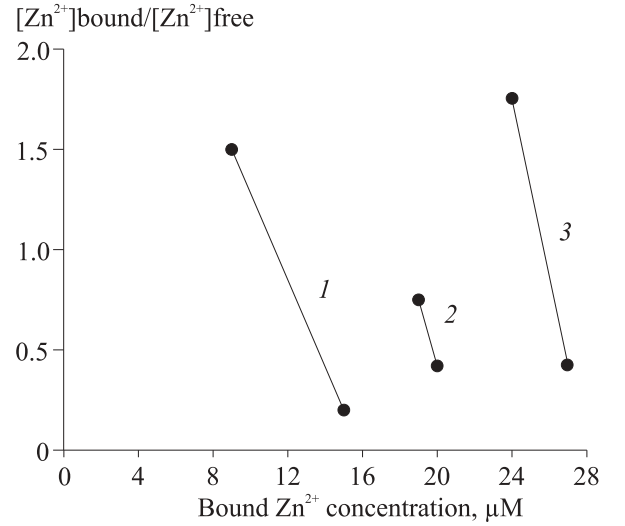

Fig. 3. Scatchard plots reflecting zinc cation binding to human serum γ-globulin. Zinc binding constants: *1*) 2.1×10^5 M^{-1}; *2*) 3.4×10^5 M^{-1}; *3*) 5.2×10^5 M^{-1}.

conditions of γ-globulin interactions with zinc cations seems to reflect unique structural organization of the protein capable of expressing sites significantly differing from each other by not only corresponding constants, but also by all physicochemical characteristics of the structure, in which the metal cation is incorporated, depending on the acquired conformation, determined, among other factors, by the status of local microenvironment.

These data confirm previously detected positive cooperative nature of the reaction [6]. Zinc binding to some amino acid residues or oligosaccharides of the protein molecule waist creates conformation prerequisites for expression of metal binding sites hidden by spatial configuration of the globule. This means that during reaction with zinc cations, similarly as with copper cations [1], γ-globulin undergoes a series of conformations and can exist in several conformations.

The estimated zinc-γ-globulin binding constants are about 10^5 M^{-1}, which attests to weak binding and instability of zinc-γ-globulin complexes. They are close to the constants describing zinc interactions with some sites of staphylococcal enterotoxin A (5×10^5 M^{-1}) [9], somewhat lower in comparison with the parameters of interactions with other proteins and single-strand fragments of IgG molecule (10^6 M^{-1}) [9,14], differ by two orders of magnitude from parameters of zinc binding sites in model peptides (4×10^7 M^{-1}) [12], and virtually coincide with γ-globulin binding constants for copper ions ($1.6\text{-}3.3\times10^5$ M^{-1}) [1]. Thus γ-globulin and hence, full-sized antibodies virtually do not differ by the zinc binding parameters from other biomacromolecules nonspecifically binding metal [9,14], while zinc cations reacting with γ-globulin exhibit properties reproducing biological activity of copper [1].

On the other hand, as was previously noted, the dynamics of metal binding and specific features in structural organization of γ-globulins and antibodies put them apart from other bioactive macromolecules [1], which is confirmed by the results of our experiments demonstrating the capacity of γ-globulin proteins bind up to 60 zinc cations per molecule. This 2-3-fold surpasses protein capacity for copper cations [1].

This circumstance could be explained by γ-globulin expression of a greater number of surface sites for zinc binding in comparison with copper. However, the estimated binding constants for these metals are very close. In addition, in accordance with our previous findings [5,6], copper (but not zinc) exhibits high activity at the external bonds of the protein molecule.

The effects of zinc manifesting during its interactions with γ-globulin by affinity for intraglobular

structures and intense incorporation in the interdomain space sites, suggest that inner sites of the protein molecule, including the hinge area and binding centers opening during conformation changes after binding of certain amounts of metal, are regions capable of binding and, presumably, retaining zinc in amounts sufficient for the realization of the registered conformation rearrangements.

This circumstance should be kept in mind during therapeutic use of zinc and zinc-containing preparations, because binding (even nonspecific) of such amounts of metal by γ-globulin in physiological and clinically used concentrations of cations [7,8,10,13] can significantly modify zinc distribution in biological fluids and thus naturally shift the actual vector of transport and metabolism of these immunoactive cations.

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