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Human Serum γ-Globulin Binds Copper Cations

E. E. Babaeva, U. A. Vorobyova, M. S. Zharkova, and S. B. Cheknyov

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Binding of copper cations to human serum γ-globulin was studied using molecular ultrafiltration. The content of free metal in the filtrate was evaluated by the reaction with sodium diethyldithiocarbamate. Conformation characteristics of the protein were evaluated by UV spectrophotometry. γ-Globulin molecule has several copper-binding sites differing by binding constants and filled one-by-one as the content of bound metal increased.

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

Copper capacity to regulate cell proliferation, attenuate activation of NF-kappa-B transcription factor and generation of free radicals, stimulate phagocytosis, provide iron transport, and induce the formation of supramolecular aggregations of γ-globulin and IgG determines the important role of this metal in immune reactions [7-9,11,13].

Physiological concentrations of copper in the plasma are 10-20 μM, the content of free cations is undetectable, and the presence of cations in cells is estimated at the level of 10^{-18} M (*i.e.* less than 1 atom per cell) [5], and therefore chelating of even solitary cations by serum proteins can markedly modify cell functions requiring the presence of copper in the microenvironment.

IgG adsorbed on solid phase binds copper cations [4], while immobilized copper binds IgG from natural sources [15]. We previously showed that γ-globulin fraction proteins interact with copper cations in solution. Obvious changes in the protein conformation manifesting (depending on copper concentration) by unfolding of the molecule into extramolecular space or by compactization of the globule indicate that the metal is bound by sites located on the surface of γ-globulin molecule or in its inter-domain space [8,9].

Binding and subsequent retention of copper by the biopolymer molecule can cause changes determined by the chemical properties of cations (toxicity of copper in supraphysiological concentrations [5,10,12], redox activity of metal capable of inducing cleavage or destabilization of biological structures binding it [1,2]) and by physicochemical changes in immunoactive serum proteins under physiological conditions (manifestation of new effector properties in the proteins which bound cations and transition of this protein into a new conformation status) [3,9].

We studied conformation changes in γ-globulin during copper cation binding and evaluated the parameters of this binding.

MATERIALS AND METHODS

Human serum γ-globulin (Serva) in 0.15 M NaCl solution (pH 7.12-7.2) with protein concentration of 100 μg/ml was used. γ-Globulin samples filtered through membrane filters with 0.45-μ pores (Millipore) for elimination of large associations were incubated for 1 h at 37°C with clarified (0.22-μ

Laboratory of Cell-to-Cell Interactions, N. F. Gamaleya Institute of Epidemiology and Microbiology. ***Address for correspondence:*** cheknev@riem.ru. Cheknyov S.B.

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pores, Millipore) aqueous copper sulfate (Merc) at the metal concentrations of 0.5-4.0 μg/ml. γ-Globulin samples incubated under the same conditions without copper sulfate served as the control.

After incubation experimental and control samples (5.0 ml) were subjected to molecular ultrafiltration (10-15 min at 300*g* and 20°C) on CF-25 cones (Amicon). The volume in the cones was brought to the initial with 0.15 M NaCl and filtration was repeated as previously. Supernatants were collected from the cones, diluted to the initial volume, and spectrophotometried in UV light at 190-320 nm with a 10-nm step in a semiautomated mode using a PU 8730 UV/VIS differentiating spectrophotometer (Phillips). Spectrophotometric control of the samples was carried out at all stages of the study.

The content of free copper in the filtrate was evaluated by the complex formation reaction with sodium diethyldithiocarbamate in a final concentration of 10^{-3} M in 0.15 M NaCl solution (pH 9.0-9.2) by spectrophotometry at λ=440 nm.

Changes in optical density and molar ratios in the solution were calculated by concentration of γ-globulin determined by spectrophotometry at λ=280 nm (extinction coefficient 0.7). Acidity of the samples was controlled by Expert-001 electronic pH-meter/ionometer (Econics-Expert).

RESULTS

Incubation of γ-globulin in the solution with suprapysiological content of copper cations led to unfolding of the protein molecule into the periglobular space, which could be regarded as a manifestation of a trend to denaturing of the initial sample [8,9]. Removal of free copper (not bound to protein) from the solution restored the spectrum of γ-globulin absorption to the status close to the control (Fig. 1). Persisting changes seemed to the due to the method used (Fig. 1).

Hence, after removal of the metal excess γ-globulin with bound copper cations acquired a conformation more close to the native one, this indicating its capacity to renaturing. These data seem to rule out the possibility of realization of copper redox activity at binding sites involved in the reaction and indicate that extramolecular unfolding of the protein during reaction with the metal was reversible.

The concentrations of copper binding to γ-globulin did not surpass 20-24 μM and were below the concentrations toxic for bacterial and human cells [5,10,12] or destabilizing biomacromolecules by many folds [2]. Hence, γ-globulin, which bound copper under conditions of metal exchange, is nontoxic and will not promote cleavage of structures (cells and biopolymers) reacting with it (if we rule out the possibility that the protein releases all bound cations simultaneously into the local microenvironment).

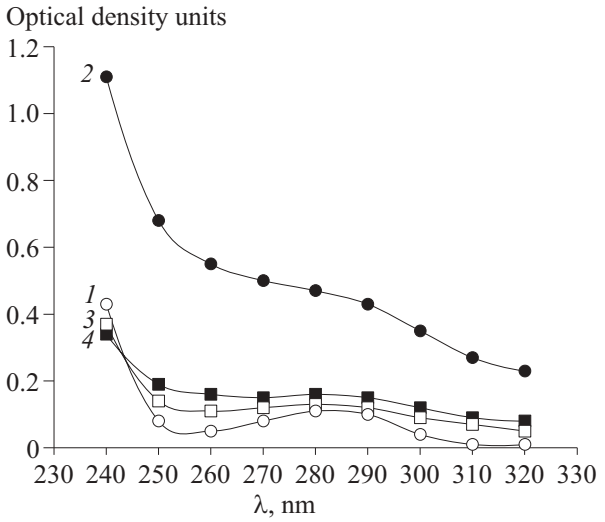

Fig. 1. UV Absorption spectra of human serum γ-globulin during binding of copper cations. Protein spectra: *1*) after 1-h incubation at 37°C in 0.15 M NaCl; *2*) after 1-h incubation at 37°C in the same solution containing aqueous copper sulfate at copper concentration of 2.0 μg/ml; *3*) reduced in 0.15 M NaCl solution after control (without copper) ultrafiltration; *4*) with bound copper and reduced in 0.15 M NaCl solution after removal of free metal by molecular ultrafiltration.

The relationship between the increment in optical density in the solution of γ-globulin with bound copper and the amount of bound metal indicates successive filling of external and intraglobular protein sites with cations. Bindings starts from external sites of γ-globulin molecule and causes its unfolding into the extramolecular space (Fig. 2). This happens before binding of the amount of cop-

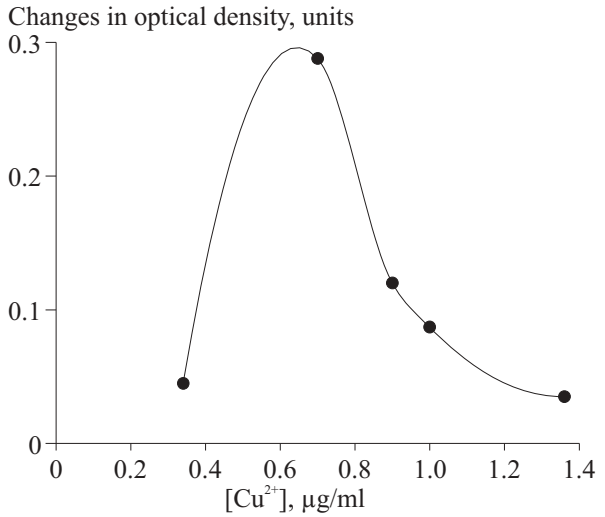

Fig. 2. Absorption increment in solution of human serum γ-globulin with bound copper cations at λ=280 nm. Abscissa: concentration of bound metal.

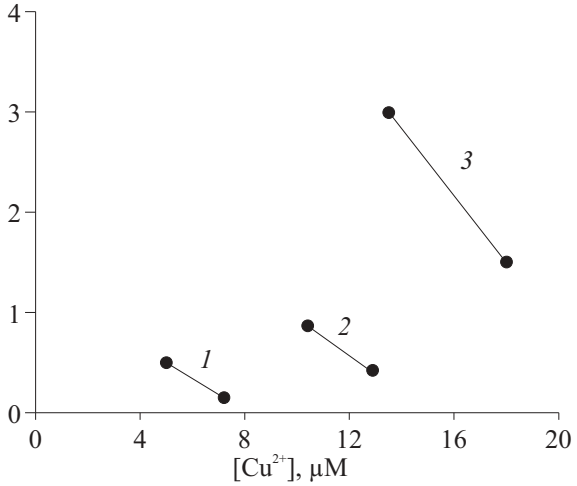

Fig. 3. Scatchard plots for copper cation binding to human serum γ-globulin. Abscissa: concentration of bound metal; ordinate: ratio of bound to free copper concentrations. Copper binding constants: *1*) 1.6×10^5 M^{-1}; *2*) 1.8×10^5 M^{-1}; *3*) 3.3×10^5 M^{-1}.

per (about 10 μM) causing the expression of previously hidden metal-binding sites. Further binding of cations seems to fill the internal regions of protein globules or the links which opened as a result of conformation changes in the molecule during copper fixation at the external sites. Optical density of the solution decreases due to natural compactization of the protein (Fig. 2). Saturation of the inner compartments of the molecule is observed after binding of about 20 μM copper (Fig. 2). The effects of the metal are then directed towards the external bonds of γ-globulin, unfolding the structure already containing copper inside the globule.

These data are confirmed by Scatchard plots detecting the presence of 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 the segments on the abscissa do not overlap, which suggest different spatial localization of the sites filled by cations one by one, with saturation of certain sites of the protein molecule with copper cations. Sharp jump-like transition from one group of sites to another indicates that copper binding centers in γ-globulin are not equivalent and mutually dependent (Fig. 3).

It seems that the jump-like conformation transitions are characteristic of copper binding sites in biomacromolecules. They were described for human ceruloplasmin, whose "non-blue" centers binding Cu^{2+} cations present as two stable forms and their conformation depends greatly on the protein microenvironment [6]. Presumably, other proteins have copper-binding centers with similar characteristics [6].

Our findings confirm previously noted positive cooperative nature of the reaction [8]. Fixation of copper by some amino acid residues or oligosaccharides of the protein molecule waist creates conformation prerequisites for expression of the metal binding sites hidden by spatial configuration of the globule. This means that reacting with copper cations, γ-globulin passes through a succession of conformations and it seems that it can be in several conformation states.

Similar changes were observed during reaction of DNA molecules with copper; transition of these molecules into a new conformation depends on the saturation of metal binding sites [3]. This transition is recorded at copper concentrations of 20-30 μM [3], corresponding, according to our findings, to the amount of metal saturating the internal compartments of γ-globulin and causing secondary unfolding of the protein molecule (initiating a typical alteration of the conformation condition).

The calculated constants of copper binding to γ-globulin are characterized by 10^5 M^{-1} order of magnitude, indicating weak binding and low stability of the complexes formed by γ-globulin with copper. For a known algorithm of comparison they are close to the constants describing copper reactions with single-stranded fragments of IgG molecule (10^6-1.7×10^6 M^{-1}) [14] and with DNA molecules in both studied conformation states ($2\text{-}4\times10^6$ and 8×10^4 M^{-1}) [3]. Hence, γ-globulin and therefore, full-sized antibodies do not differ in principle by the binding parameters from other biomacromolecules nonspecifically binding metal [3,4].

On the other hand, this time course of binding, specific features in the structural organization of γ-globulins and antibodies creating prerequisites for expression of external and internal copper binding sites by the molecules (the inner sites seem to be located in the "hinge" area, characterized by less compact polypeptide chain packing) and the capacity of γ-globulin to bind up to 20-30 copper cations per molecule calculated in our study make these proteins special in comparison with other bioactive macromolecules.

γ-Globulins are traditionally not regarded as active or specialized copper carriers. Obviously, after fixation of copper cations the proteins will with high probability donate some cations to other biomacromolecules or to cells expressing more active metal chelators on the membrane.

These data experimentally confirm the capacity of γ-globulins and antibodies to participate in metal exchange processes, normally taking place in the lymphocyte microenvironment, while the proteins proper can be regarded as factors of cellular activity regulation, needing metal cations for realization of specialized functions.

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