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Biphasic Interaction of γ-Globulin with Copper Cations

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Interactions of human serum γ-globulin with copper cations in solution were studied by differential ultraviolet spectrophotometry. Copper in supraphysiological concentrations increases optical density of protein solution, reflecting the effect of γ-globulin saturation with metal. In physiological and lower concentrations of copper cations we observed hypochromia in the protein absorption spectrum. Conformational changes in γ-globulin molecule during interactions with copper by the surface and intramolecular binding sites and possible role of bivalent metal cations in the maintenance of certain conformations of immunoactive serum proteins are discussed.

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

The role of copper cations in the maintenance of organism's resistance to infections, important biochemical processes, regulation of phagocytic activity of neutrophils and proliferation of T-lymphocytes, iron transport and metabolism is well known [5,9,12]. Copper incorporation by cells and delivery of cations into specialized cell compartments are regulated by at least 4 genes [9]. Metal transport in biological fluids and tissues is realized by serum and tissue forms of ceruloplasmin (CP). In mammalians the greater part of copper ions is bound to CP.

Albumin ranks second among copper transporter [5], while serum globulin fraction proteins, specifically, γ-globulins, little contribute to cation binding and transfer due to high rigidity of their molecular structure and compact spatial packing of the globule.

There are data on the expression of metal-binding sites in proteins of γ-globulin fraction, on the presence of bound copper in these biomacromolecules, and realization of some intermolecular interactions involving γ-globulins and IgG through copper cation exchange [2,3,14,15].

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Our findings indicate that γ-globulins can expose carbohydrate-rich components of the molecule and unfold them into extramolecular space [3]. Polysaccharide (polyanion) chains in this form can effectively bind metal, thus regulating its content in cell microenvironment. It is known that cell activity depends on the presence of certain cations in the perimembrane space. Since carbohydrate-rich components of γ-globulin can be released during normal intermolecular interactions [3] and the metal forms some intramolecular noncovalent bonds in the structure of complex protein molecules, γ-globulins seem to be proteins capable of binding or donating cations, depending on the environmental conditions, in other words, serve as transport proteins [3].

We evaluated conformational changes in the protein and concentration dependence during interactions of γ-globulin with copper cations. In contrast to metal binding to IgG adsorbed on protein-A-sepharose-packed column [2], our experiments were performed under near-physiological conditions.

MATERIALS AND METHODS

We used human serum γ-globulin (Serva) dissolved in 0.15 M NaCl (pH 7.15-7.2) to concentrations of 50, 100, 150, and 200 μg protein /ml. Samples free from

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large protein aggregates, filtered through membrane filters (0.45-μ pores, Millipore), were incubated for 1 h at 37°C with aqueous copper chloride (II) used at copper concentrations from 0.65 ng/ml to 4 μg/ml. The reaction was carried out in conical graduated polystyrene 10-ml tubes (Costar); the volume of each sample was 5 ml. Samples of γ-globulin incubated with aqueous copper sulfate (II) and samples containing no copper salts served as the control. Control tests were carried out using the same protein and cation concentrations as in experiments with copper chloride.

The reaction was evaluated by UV spectrophotometry at 190-320 nm with a 10-nm step in a semiautomated mode on a PU 8730 UV/VIS differential spectrophotometer (Phillips). Changes in optical density and molar ratio in the solution, represented by the curves, were calculated basing on γ-globulin concentrations determined by absorption at 280 nm (extinction coefficient 0.7). Acidity was controlled with an electron pH-meter Expert-001 (Econics-Expert). Experiments with individual concentrations of copper salts were repeated twice or thrice.

RESULTS

Normally, copper content in human serum is 10-20 μM, mean concentration 1 μg/ml). Since copper is virtually completely bound to CP, free cation concentration can be close to or below 0.15-0.75 μM (0.01-0.05 μg/ml), and it seems that this content should be regarded as physiological.

The effects of supraphysiological copper concentrations manifest in an optical density increment in γ-globulin absorption spectrum. The absorption increased for the entire protein spectrum with the most pronounced increment at 250-260 nm, including phenylalanine chromophore absorption band. Differential UV spectra revealed changes caused by rearrangement of bonds responsible for packing of polypeptide chains and formation of a metal complex with charge transfer.

The study of γ-globulin interactions with copper cations in concentrations of 0.1-4.0 μg/ml showed a characteristic relationship between optical density increment and copper content in the sample (Fig. 1.).

The type of the detected relationship and the results of the analysis of differential and subtraction UV spectra suggest that copper in concentrations surpassing the content of free cations in normal serum saturates the ligands on the protein molecule surface (lateral groups of amino acids, mono- and oligosaccharide residues, specialized sites for metal binding). This reduces the compactness of the protein globule and causes its unfolding into the extramolecular space. Simultaneously some carbohydrates and amino acids located in the interdomain area of γ-globulin are exposed into the water phase. These latter can include phenylalanine hydrophobic residues responsible for the detected shift in γ-globulin differential spectrum (near 260 nm).

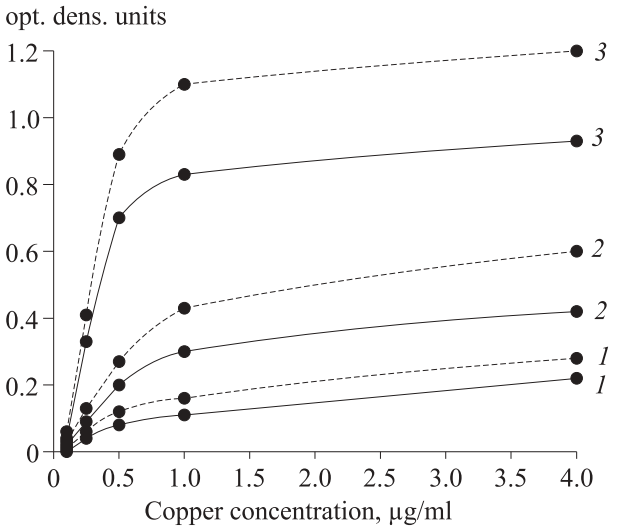

Fig. 1. Increment in the optical density of human serum γ-globulin in the presence of supraphysiological copper concentrations. Evaluated at λ=280 nm (continuous lines) and 260 nm (intermittent lines). γ-Globulin concentrations: *1*) 50; *2*) 100; *3*) 200 μg/ml.

Exposure of these hydrophobic protein fragments into the extramolecular space increases the probability of intermolecular hydrophobic interactions and promotes the formation of supramolecular γ-globulin aggregations. We previously showed that the intensity of aggregation at copper concentrations of 0.25-4.0 μg/ml depended on the content of metal cations in the sample, this allowing us to consider γ-globulin aggregation as a metal-dependent process [4].

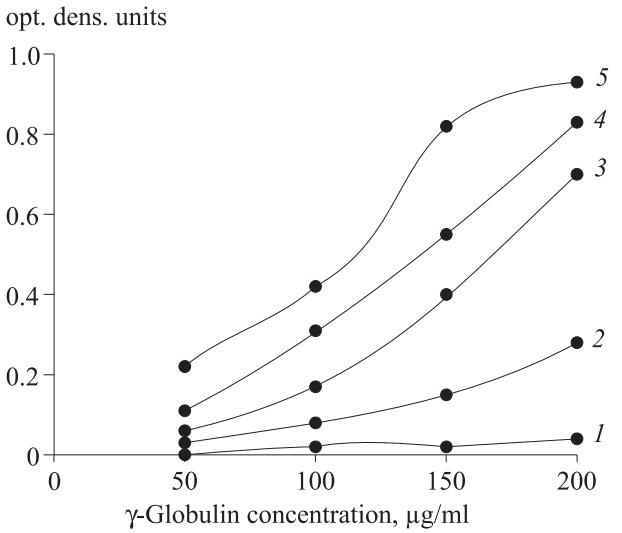

Fig. 2. Relationship between the increment in the optical density of γ-globulin solution during interaction with copper cations and protein concentration in the sample. Evaluated at λ=280 nm. Copper concentrations: *1*) 0.1; *2*) 0.25; *3*) 0.5; *4*) 1.0; *5*) 4.0 μg/ml.

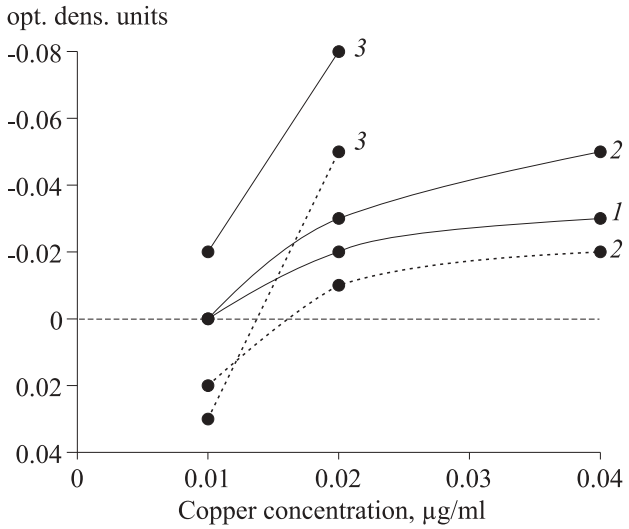

Fig. 3. Hypochromia of γ-globulin absorption spectrum in the presence of copper cations. Evaluation at λ=280 nm (continuous lines) and 260 nm (dotted lines). γ-Globulin concentrations: *1*) 50; *2*) 100; *3*) 200 μg/ml.

It cannot be excluded that copper contributes to reduction of disulfide bonds in the waist region of the protein molecule. Reduced sulfhydryl groups form coordination bonds with oxidized metal form, where the metal acts as a bridge between the molecules of *in situ* reduced γ-globulin.

The increment in the optical density of γ-globulin solution in the presence of 0.1-4.0 μg/ml copper is characterized by exponential dependence on the protein content in the sample (Fig. 2). The results attest to cooperative interaction: binding of copper cations by some amino acid residues creates conformational prerequisites for exposure of more and more sites capable of reacting with metal into the extramolecular space.

Analysis of the data suggests that reaction between γ-globulin and copper fits the kinetics of enzymatic reactions and substantiates interpretation of the studied interactions from the viewpoint of specific binding of metals by sites located on the surface of the protein globule.

The use of copper in concentrations close to physiological and lower causes hypochromia of γ-globulin absorption spectrum recorded in the long-wave band (260-280 nm), paralleled by a 220-nm maximum in the subtraction UV spectra. Our experiments demonstrated spectral shifts indicating incorporation of the metal in the inner compartments of the protein globule with subsequent formation of intramolecular coordination bonds in the hinge area. Compactization of protein, natural under these conditions, reduces the degree of molecule unfolding into the periglobular space.

The interactions of γ-globulin with copper cations under these conditions also demonstrate protein saturation with metal (Fig. 3). However, this regularity is obvious not for all concentrations of γ-globulin used in the study. This can be explained as follows: since the interactions are cooperative, it is impossible to rule out the effect of sharp transfer of the reaction to the external sites of metal binding at concentrations used in the study (*i.e.* copper/protein concentrations close to the serum molar concentration).

Copper binding by intramolecular sites of γ-globulin coordinating the metal, similarly as interactions on the globule surface, is described by enzymatic reaction kinetics and can promote detection of specialized copper binding sites located in the interdomain space.

It was previously shown that immunoglobulins express metal binding sites in the conserved [14] and variable region of the molecule [13,15]. Histidine or tryptophane residues participate in the formation of copper binding sites [13,15]. In some proteins cystein also participates in the formation of sites for binding transitional metals (including copper) [8].

Copper-binding sites are widely presented on biomacromolecules [10]. In antibodies the complementary sites of variable regions form 11 sites for copper and zinc binding [15]. Lectin, heparin, IgG molecules [3], and some plasma and tissue proteins and glycoproteins bind copper cations [7,8]. Therefore, copper cation exchange seems to be an important factor of intermolecular interactions, which can appreciably modify the conformation and characteristics of biopolymers.

Indeed, copper binding essentially modifies the effector functions of macromolecules. Due to unique stereochemistry, copper binding prevents the formation of calcium binding site in Con A. Chelated copper inhibits neurotensin binding to brain cell membranes and its contractile effect [8]. The presence of copper in nanomolar concentrations intensifies the reaction between histidine-proline-rich plasma glycoprotein and heparin, increases the protein affinity for heparin, and shifts the stochiometry of this interaction from 1:1 to 1:2 [7].

Since copper cations are the most active bivalent metal cations by the strength of forming bonds for almost all ligands, they can compete for binding sites in biomacromolecules and hence, for the regulation of the content and, therefore, selection of metals in the extramolecular space. This seems to be important, if we recollect the relationships and mutual dependence of the effects mediated by metal cations in biological systems [1,11].

The results of this study demonstrate a biphasic pattern of γ-globulin interactions with metal cations and show that metal binding to protein is realized in external or intraglobular sites. Since our experiments

were carried out under conditions approximating physiological, we can hypothesize the appearance of surface and internal protein-copper complexes during normal intermolecular interactions. Since these complexes differ appreciably by availability of copper binding sites, they can significantly differ by parameters of interactions with copper (and probably by the dissociation constant), determined, in turn, by many external factors.

Therefore, if chelation of copper cations by γ-globulin and occupation of protein metal binding sites will be realized during some intermolecular contacts stage-by-stage (in accordance with physical chemistry laws), the appearance of free binding sites after cation donation by protein should be also biphasic. Then, a time period naturally exists, during which some sites occupied by bound copper will retain sufficiently stable structure and serum γ-globulin fraction proteins will transport copper, at least in the local environment.

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