MICROBIOLOGY AND IMMUNOLOGY

Antigenic Specificity of Human Serum γ-Globulin Samples Obtained under Conditions of Equimolar Binding of Copper and Zinc Cations

S. B. Cheknyov, E. E. Babayeva, E. A. Denisova, U. A. Vorobyova, and E. M. Mongush

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 2, pp. 170-174, February, 2007
Original article submitted August 10, 2006

Samples of human serum γ-globulin modified by equimolar binding of copper and zinc cations were obtained using the method of molecular ultrafiltration. Conformation characteristics of the protein were studied by UV spectrophotometry. Immunochemical study included radial immunodiffusion test, direct and sandwich enzyme immunoassays. Conformation changes in γ-globulin caused by incorporation of solitary metal cations into the protein molecule modified presentation of antigenic determinants on the globule surface and their availability for recognition by specific antibodies. New antigenic determinants and new antigenic specificity of γ-globulin are not formed under these conditions.

Key Words: *γ-globulin; zinc; copper; equimolarity; antigenic activity*

Equimolar interactions of human serum γ-globulin with copper and zinc cations reproduces physiological processes of metal exchange between plasma macromolecules and helps to evaluate conformation changes in the protein essential for humoral and cellular immunity reactions. This is seen from the following proportions typical of normal human serum: 1-3 copper cations per 4 γ-globulin molecules and 1 zinc cation per 1 protein molecule.

These concentrations enable realization of the regulatory effects of metals most important for intermolecular interactions and cell systems [6,7,9,10, 13-15]. Since the absolute majority of cations in circulation are bound to proteins, glycoproteins, and other plasma components [6,7,9,13], there are good grounds to expect that copper toxicity towards body cells does not manifest under physiological conditions [1], no metal-dependent protein aggregation takes place [2,5], and spontaneous aggregation is inhibited [7]. Cations can incorporate into the macromolecule structure [2,3,6,12,13], modify their conformation [2,3,6,9]; they also can penetrate into cells and bind to specific chaperones thus regulating the function of metal-dependent proteins [1]. Metal chelating can modify the results of serological tests in diagnostic practice [12].

We previously showed that loading of γ-globulin proteins with molar excess of metals causes pronounced conformation changes in γ-globulin molecule, modifies presentation of antigenic determinants on the globule surface and their availability for recognition by specific antibodies, thus leading to overestimation in Mancini test [4].

The aim of our study was to obtain samples of human serum γ-globulin bound with equimolar quan-

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

0007-4888/07/1432-0210

tity of copper and zinc cations and their conformation and antigenic characterization.

MATERIALS AND METHODS

Human serum γ-globulin (ICN) in 0.15 M NaCl (pH 7.15) with protein concentration of 100 μg/ml was used. Large associations were removed by filtering through 0.45-μ membranes (Millipore). γ-Globulin samples were incubated at 37°C for 1 h with aqueous copper sulfate (Merc) or zinc chloride (metal concentration 0.25 μg/ml). γ-Globulin samples incubated under the same conditions without metal salts served as the control.

After incubation protein samples (7.0 ml) were subjected to molecular ultrafiltration on CF-25 cones (Amicon) at 300*g* and 20°C for 10-15 min. The volume in the cones was brought to the initial one with 0.15 M NaCl and filtration was repeated in the same regimen. The supernatants were removed from the cones, the volume of the samples was brought to the initial one, and UV spectrophotometry was carried out at λ=190-320 nm at a 10-nm step in a semiautomated mode using PU 8730 UV/VIS differentiating spectrophotometer (Philips).

The content of free metals in the filtrate was evaluated spectrophotometrically by the reaction of complex formation (copper with sodium diethyldithiocarbamate at λ=440 nm, zinc with o-phenanthroline at λ=226 nm).

Radial immunodiffusion tests after Ouchterlony and Mancini were carried out by standard methods in 0.75% agarose gel (Sigma) with native γ-globulin (ICN; initial concentration 100 μg/ml) as the test antigen. Goat antibodies to human IgG (H+L) (Medgamal) in a concentration of 0.5 vol.% were used in Mancini test and in 1:10 dilution in Ouchterlony test. Peroxidase-labeled rabbit antibodies to human IgG (H+L, Medgamal) were used in enzyme immunoassay. Orthophenylene diamine (0.05% solution) in 0.5 M citrate buffer served as the substrate; the reactions were evaluated using ELISA Processor II (Behring).

Protein concentration and molar ratios in the solution were estimated spectrophotometrically (λ=280 nm, extinction coefficient 0.7). Acidity of the samples was measured by Ekspert-001 electron pH-meter/ionometer (Econics-Ekspert).

RESULTS

Samples of human serum γ-globulin containing one copper or zinc cation per protein molecule were obtained.

Differential spectrophotometry showed compactization of the macromolecule (Fig. 1) at the expense of incorporation of solitary cations into the intraglobular compartments, formation of a field of metal-stabilized ligands, and increased orderliness of intramolecular structures. Due to previously revealed high tropism of zinc cations to the inner regions of γ-globulin [2,3], hypochromy of the spectrum in near UV band was more pronounced for zinc-modified protein (Fig. 1). γ-Globulin absorption band in far UV band changed negligibly under conditions of protein charge by molar excess of zinc, while copper caused hypochromy [4]. Absorption spectra of protein which bound one copper and one zinc cations per molecule virtually coincided in the short-wave band (Fig. 1).

Loading of the protein with the excess of copper cations markedly changed spatial packing of γ-globulin molecule [4]. The distribution of antigenic determinants of protein changed significantly: they were not detected in radial immunodiffusion tests [4]. Equimolar binding modified the expression of antigenic determinants to a significantly lesser degree. The equivalence zone was clearly seen and corresponded to that of control γ-globulin. Solitary zinc cations acted similarly. γ-Globulin with bound zinc cations formed better structured precipitation bands in Ouchterlony test in comparison with protein modified by molar excess of metal [4].

The involvement of copper and zinc cations in the formation and stabilization of intermolecular contacts [1,2,4,14] manifested in Mancini test. γ-Globulin samples modified by both cations gave overestimated results. Previously detected expression of antigenic determinants (hidden in the native con-

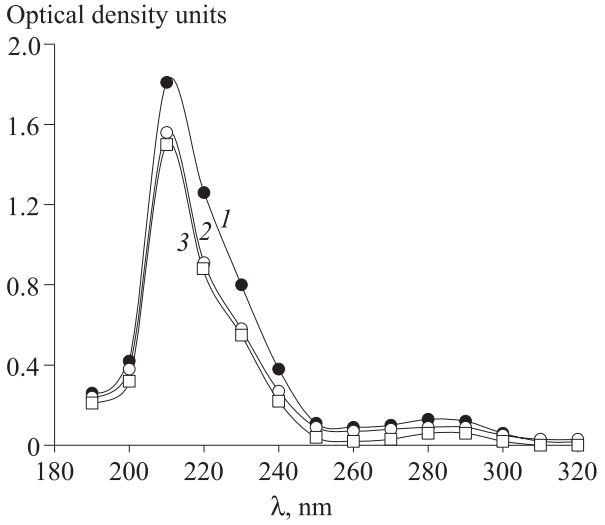

Fig. 1. UV absorption spectra of control (*1*) and copper- (*2*) and zinc-modified (*3*) human serum γ-globulin samples. The absorption spectrum of control protein is presented by the results of two independent experiments.

formation) the protein loaded with molar zinc excess proved limited potentialities of Mancini method as a quantitative test for clinical practice [4]. These limitations were seen in our study as well. They were probably determined by the capacity of γ-globulin fraction proteins to chelate solitary metal cations from the microenvironment, undergo conformation changes because of their incorporation in the inner regions of the molecule, which modified the distribution of specific antigenic determinants, and to form metal-stabilized supramolecular complexes.

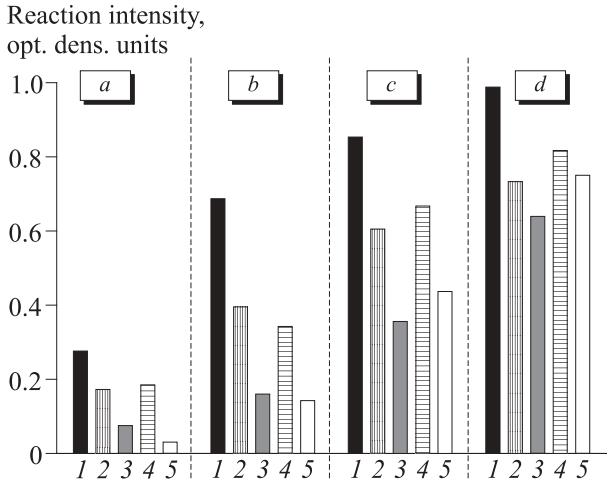

Fig. 2. Immunochemical characteristics of copper- and zinc-modified γ-globulin in direct EIA. Protein concentration: *a*) 1.0 μg/ml; *b*) 2.5 μg/ml; *c*) 5.0 μg/ml; *d*) 10.0 μg/ml. γ-Globulin samples: *1*) native; *2*) control for zinc; *3*) control for copper; *4*) zinc-modified; *5*) copper-modified. Conjugate dilution: 1:1000.

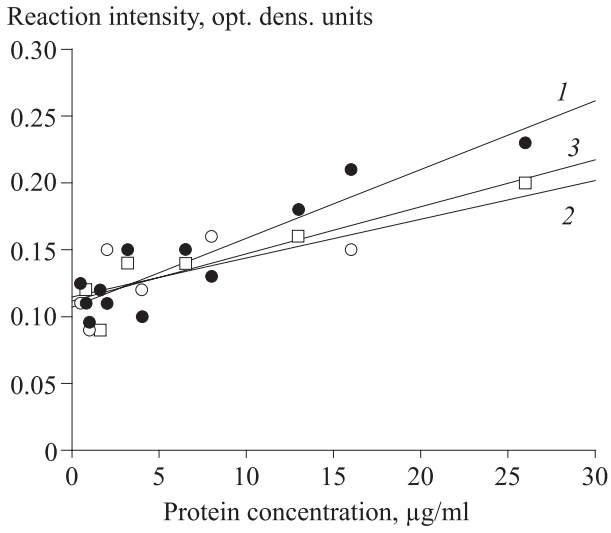

Fig. 3. Immunochemical characterization of copper- and zinc-modified γ-globulin in sandwich EIA. *1* and dark circles: control γ-globulin; *2* and light circles: zinc-modified γ-globulin; *3* and squares: copper-modified γ-globulin. Conjugate dilution: 1:500. Control protein values are presented as the means of control protein samples for copper and zinc.

Immunochemical characterization of modified γ-globulin in direct EIA showed that binding of solitary cations modulated polarization of Fc-fragment groups (at the expense of which solid phase is formed) to a far lesser degree than under conditions of their molar excess, while the effects of copper and zinc stabilizing γ-globulin Fc-region were observed at protein concentrations of 5.0 and 10.0 μg/ml (Fig. 2). The intensity of reaction with specific antibodies did not reduce in the majority of cases for zinc- and copper- modified γ-globulin in comparison with control protein; in some cases it even increased (Fig. 2).

Discrete increase in sample concentration led to an increase in the reaction intensity (1.7 times in tests with zinc and 3.2 times in tests with copper, Fig. 2). The corresponding values for control proteins were 1.7 and 2.1, for native γ-globulin 1.6 times (Fig. 2). A peculiar feature of copper manifests by low reaction of antibodies with determinants of protein under conditions of incomplete monolayer (Fig. 2).

The results of direct EIA indicate the absence of pronounced changes in the presentation of specific antigenic determinants on the surface of γ-globulin molecule with bound solitary metal cations, which is a specific feature of equimolar interactions of the protein with copper and zinc, in contrast to interactions with molar excess of cations [4].

The data of sandwich EIA in general confirm similarity of antigenic characteristics of metal-modified and control γ-globulin (Fig. 3). On the other hand, these data indicate that copper and zinc cations incorporated into the inner compartments of the protein molecule and making the globule more compact also promote translocation of some antigenic determinants from γ-globulin surface and their shielding with lateral amino acid residues. The slope of the curve describing the recognition of γ-globulin with bound copper decreases 1.7 times in comparison with the control protein curve and 2.5 times with the curve for protein with bound zinc (Fig. 3), which confirms previously detected zinc tropism to intraglobular structures of the protein [2,3].

Antigenic groups loosing contact with extramolecular environment and submerging into the depth of the molecule obviously became more conservative compared to groups located on the surface of γ-globulin and available for intermolecular contacts. It is with good grounds that in theory of protein structure and dynamics metal cations are regarded as a factor of evolutional "editing" of protein structure, the functions of protein being determined by a limited number of amino acid residues reacting with metal.

Similarity of the effects of solitary copper and zinc cations incorporated in γ-globulin molecular structure detected by all the methods used in the study exhibits structural similarity of metal binding sites, their location in the same regions of protein molecule, virtually complete similarity of intramolecular complexes forming during interactions between these metals and γ-globulin.

The specific features of these interactions should be borne in mind when evaluating the regulatory effects of copper and zinc, supporting the main immunopoiesis and immunogenesis processes, involving metal cations as the supporting and regulatory factors.

Copper serves not only as a potent oxidizer and toxic cation in biological systems [1,4], but also provides normal development of the myeloid and lymphoid hemopoietic stems [15] and realization of anti-infection defense mechanisms [10]. Copper deficiency is associated with inhibited transcription of IL-2 gene and hence, with its reduced production this cytokine and the resultant reduced proliferation of T-lymphocytes [8,11].

Zinc is not only a stabilizer of cell membranes and an antioxidant [4,14]. It determines the biological essence of thymulin [13], provides the development and functioning of natural and acquired immunity systems [14], regulates the cytotoxic and proliferative reactions of lymphocytes, production of antibodies and a cascade of immunoactive cytokines, macrophage functions [13,14]. Zinc deficiency is associated with reduced infection resistance and potentiation of apoptosis [13,14].

These data indicate that conformation changes in γ-globulin caused by incorporation of even solitary copper and zinc cations in the protein molecule structure can modify the presentation of antigenic determinants on the globule surface and their availability for recognition and binding by specific antibodies. On the other hand, no signs of formation of new antigenic determinants of protein and formation of new antigenic specificity, which might induce the production of antibodies to own γ-globulin, were detected under conditions reproducing the normal metal exchange processes between plasma macromolecules.

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