Formation of Supramolecular Aggregations of γ-Globulin as a Metal-Dependent Process

S. B. Cheknev, E. E. Babaeva, M. S. Zharkova, A. A. Poroshina, U. A. Vorob'yova

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The presence of copper cations in the solution of human serum γ-globulin induced the formation of supramolecular forms of the protein. The intensity of this reaction increased with increasing copper concentration. The mechanisms of γ-globulin aggregate formation under normal conditions and the possible role of bivalent metal cations in the regulation of protein effector functions are discussed.

Key Words: *γ-globulin; aggregate; metal*

Aggregated form of human serum γ-globulin stimulates lymphocyte blast transformation and suppresses activity of natural killer cells [6]. This phenomenon is probably determined by the effects of carbohydrate-rich components of proteins exposed from the native globule during aggregation [5] and markedly differing by many characteristics from the initial material [2].

Aggregation of IgG is associated with acquisition of a new antigenic specificity by the molecule due to the appearance of new antigenic determinants [1], which, in turn, results from conformation changes in the hinge region of the molecule [4] with subsequent involvement of "adhesive" Fc fragments in this process. For the most demonstrative manifestation of the new antigenic characteristics their number should amount to 6 [1].

Aggregation of IgG significantly modulates the effector characteristics of the protein: stimulates its complement-fixing activity [4], determines effector reactions with FcγRII and FcγRIII on neutrophils with subsequent clustering and triggering of the cell biological response [9,12], and increases the number of metal binding sites at the expense of rearrangement of non-covalent bonds of semicystine residues in the hinge region [3].

Despite the fact that the formation of supramolecular forms of serum proteins can play an important role in antigen—antibody interactions, modulate vascular permeability, and be a factor of development of some immunopathological reactions, the mechanisms of aggregation under conditions approaching the normal are virtually not studied.

Here we evaluated the intensity of γ-globulin aggregations in the presence of copper ions.

MATERIALS AND METHODS

Human serum γ-globulin (Serva) was dissolved in 0.15 M NaCl solution (pH 7.15-7.25) to protein concentrations of 50, 100, 150, and 200 μg/ml. Large protein associations were removed by filtering through Millipore filters (0.45 μ) and the samples were incubated for 1 h at 37°C with copper sulfate or copper chloride (II), copper concentrations of 0.01-4.00 μg/ml. Aggregation was studied at 20°C. The reactions were carried out in conical graduated polystyrene 10-ml tubes (Costar). The volume of each sample was 5 ml. Control samples contained no copper salts.

The reaction was evaluated visually and differentiated as follows: transparent solution, opalescence, suspension, precipitate (presence and volume). Expe-

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. Cheknev

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riments with each concentration of copper salts were repeated twice or thrice.

The state of protein in the solution was monitored by UV spectrophotometry (λ=190-320 nm). Molar ratio was calculated from γ-globulin concentrations evaluated by absorption at λ=280 nm (extinction coefficient 0.7).

RESULTS

In the presence of 0.25-4.00 μg/ml copper sulfate we observed the formation of supramolecular aggregates of γ-globulin (appearance of suspension or precipitate of up to 0.3 ml, Table 1). The reaction intensity increased with increasing copper content for all studied protein concentrations.

Copper chloride caused more pronounced aggregation (Table 2). The volume of visible precipitate reached 0.5 ml. The intensity of aggregation increased with increasing copper concentration in the sample.

No visible changes were observed in samples incubated at 20°C. No aggregation of γ-globulin was observed in control samples containing no copper, both at ambient temperature and at 37°C.

In our test system γ-globulin formed aggregate only in the presence of copper salts after 1-h incubation at 37°C. Similar results of protein interactions with hydrated copper chloride and sulfate (II) characterized by principally different spatial configuration and the arrangement of an appreciable number of metal binding sites in the structure of γ-globulin confirm that the detected effects are determined by the presence of copper, while the formation of supramolecular forms of the protein can be regarded as a metal-dependent process.

It is known that some membrane IgM on murine B cells are organized into $(H:L)_n$ complexes, where is 3, 4, or 5 [7]. The possibility of formation of these structures can be explained by the formation of intermolecular disulfide bonds. The authors note that IgM in large aggregations should possess higher avidity for antigen and bind it at lower concentrations [7].

Aggregated IgA (most markedly, in complexes, consisting of 5-6 molecules) many-fold increases the production of IL-2 by transformed murine B cells [10], while in the membrane-associated form it triggers the same response (oxidative burst) of normal human neutrophils as IgG [12]. The observed synergism between IgA and IgG FcR suggests that IgA even in low concentrations can appreciably potentiate opsonizing or inflammatory IgG response [12].

Association of rheumatoid factor IgG mediated via galactose residues of Fab fragments oligosaccharides is believed to be one of the mechanisms of the development of chronic inflammatory changes in the immunopathogenesis of rheumatoid arthritis [11]. This fact confirms similarity between this disease and pulmonary tuberculosis and suggests the existence of common mechanisms of immune disorders triggered by the infectious agent [11].

TABLE 1. Formation of γ-Globulin Aggregates in the Presence of Hydrated Copper Sulfate (II) after 1-h Incubation at 37°C

Protein concentration, μg/ml	Copper concentration, μg/ml			
	0.25	0.5	1.0	4.0
200	±	±±	++++	++++++
150	±	±	++	++++
100	—	—	+	++
50	—	—	±±	+

Note. Here and in Table 2: "—" transparent solution; "±" suspension; "±±" and more — appreciable increase in suspension opalescence; "+" 0.05 ml precipitate; "++" greater volume of precipitate, with a 0.05-ml step.

In the present work the reaction between copper ions and γ-globulin was studied in a wide range of molar ratios: from 1 Cu^{2+} per 5-10 γ-globulin molecules to almost 300 Cu^{2+} per protein molecule, *i. e.* under conditions of molar insufficiency and appreciable excess of metal ions. In normal human serum the proportion between Cu^{2+} ions and γ-globulin molecules is approximately 1:4. The first manifestations of supramolecular forms of γ-globulin in the solution were observed at a ratio of 3-5 cations per protein molecule (Tables 1, 2).

The formation of large insoluble aggregations of rat monoclonal IgG1 occurs in the presence of at least 4 Cu^{2+} per protein molecule [8]. Even 10-fold excess of copper ions (not physiological number of cations per protein molecule) does not reduce antigen-binding activity of antibodies [8].

We visually observed aggregation at copper concentrations surpassing the physiological, as almost 95% copper in mammalians is integrated in serum or tissue ceruloplasmin (Tables 1, 2). Some observations (8 of 20) indicated weak or appreciable opalescence of the solution in the presence of 0.01, 0.02, 0.04, and

TABLE 2. Formation of γ-Globulin Aggregates in the Presence of Hydrated Copper Chloride (**II**) after 1-h Incubation at 37°C

Protein concentration, μg/ml	Copper concentration, μg/ml			
	0.25	0.5	1.0	4.0
200	—	+	++++++	++++++++++
150	—	+	++++++	++++++++++
100	±	±±	±±±	±±±±
50	—	±	±	±

0.10 μg/ml copper chloride, but not sulfate. These results were observed with cation concentrations not only below the physiological or estimated with consideration for copper binding to ceruloplasmin, but in the range of ratios approaching the serum molar one.

Since copper ions (II) are the most active among bivalent metal cations by the strength of formed bonds for almost all ligands, our results suggest that under certain conditions these ions can be essential for the course of immune reactions, causing the formation of supramolecular forms of γ-globulin, antibodies, and other proteins in the serum and on the surface of cell membranes and essentially modulate the functions of these biomacromolecules.

REFERENCES

1. A. Ya. Kul'berg, *Antiimmunoglobulins* [in Russian], Moscow (1978).
2. A. Ya. Kul'berg, E. E. Babaeva, and A. A. Rodnikova, *Immunologiya*, No. 4, 12-14 (1994).
3. A. Ya. Kul'berg and Yu. B. Berkun, *Ibid.*, No. 3, 21-24 (1999).
4. V. S. Khristoforov, V. M. Persanov, A. G. Sukhomudrenko, *et al.*, *Biofizika*, **32**, No. 1, 32-36 (1987).
5. S. B. Cheknev, *Byull. Eksp. Biol. Med.*, **128**, No. 11, 545-547 (1999).
6. S. B. Cheknev, Ya. G. Ashmanova, E. E. Babaeva, *et al.*, *Ibid.*, **118**, No. 12, 625-630 (1994).
7. S. Alhason, R. Santini, Q. Li, and A. Rosenspire, *Mol. Immunol.*, **30**, No. 17, 1593-1599 (1993).
8. R. Bauer, A. Muller, M. Richter, *et al.*, *Biochim. Biophys. Acta*, **1334**, No. 1, 98-108 (1997).
9. W. D. Jr. Blackburn and W. W. Chatham, *J. Leukoc. Biol.*, **55**, 793-797 (1994).
10. T. J. Reterink, G. van Zandbergen, M. van Egmond, *et al.*, *Eur. J. Immunol.*, **27**, 2219-2224 (1997).
11. I. M. Roitt, R. A. Dwek, R. B. Parekh, *et al.*, *Recenti Prog. Med.*, **79**, Nos. 7-8, 314-317 (1988).
12. W. W. Stewart, R. L. Mazengera, Li Shen, and M. A. Kerr, *J. Leukoc. Biol.*, **56**, 481-487 (1994).