CORRELATION BETWEEN THE STRUCTURE OF IgG AND ITS Fc-FRAGMENT AND THEIR ABILITY TO INTERACT WITH STAPHYLOCOCCAL PROTEIN A

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Interaction between 7S monomers of rabbit IgG, dimers of molecules of this protein, IgG with disulfide bond ruptured in the hinge region of the molecule, and various fragments of IgG molecules, on the one hand, and protein A of *Staphylococcus aureus*, on the other hand, were investigated by the passive hemagglutination inhibition test. Only the Fc-fragment of the rabbit IgG molecule obtained with papain was shown to bind protein A. Activity of the Fc-fragment on a molar basis was shown to be only one-sixth of that of native IgG. After repair of the disulfide bond between the chains in the hinge region of the IgG molecule, its ability to bind protein A was reduced by two-thirds. The binding activity of IgG was increased on a molar basis twelvefold as a result of its spontaneous dimerization. It is concluded from the results that the structural organization of the Fc-fragment of the IgG molecule correlates with its ability to interact with protein A.

KEY WORDS: rabbit immunoglobulin G (IgG); IgG dimers; proteolytic fragments of IgG; interchain disulfide bond in the hinge region of the IgG molecule; interaction with protein A of *Staphylococcus aureus*.

Immunoglobulin G (IgG) obtained from various species is known to interact with protein A of *Staphylococcus aureus*. The complexes thus formed have a low dissociation constant and have the characteristic properties of antigen–antibody complexes. The binding sites for protein A are located in the Fc-fragment of the IgG molecule [8]. However, neither the C-terminal half of the Fc-fragment (the $C_\gamma 3$ domain) nor the isolated NH_2-terminal halves of this fragment ($C_\gamma 2$ domains) of human IgG react with protein A [6]. This may mean that contact between the domains composing the Fc-fragment and a definite mutual orientation of these domains are essential for binding protein A.

The object of this investigation was to study what features of the structural organization of the IgG molecule and its Fc-fragment are important for interaction with protein A. For this purpose interaction of protein A with rabbit IgG, with various fragments of the molecules of this protein, with dimers of IgG molecules, and also with IgG in which the disulfide bond in the hinge region of the molecules was reduced and alkylated, was investigated.

EXPERIMENTAL METHOD

Protein A was isolated from the culture medium of strain *Staph. aureus* A-676* by affinity chromatography [7]. IgG was isolated from immune rabbit sera by ion-exchange chromatography on DEAE-cellulose [13]. The 7S monomers of this protein and its 9.6S dimers were obtained by gel-filtration on Sephadex G-200 in 0.15 N NaCl, 0.01 M Tris-HCl, pH 7.8. The Fab- and Fc-fragments of IgG were isolated from a papain digest of this protein by ion-exchange chromatography on CM-cellulose [12].

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TABLE 1. Inhibiting Activity of Rabbit IgG Preparations and Fragments of Its Molecule in the Passive Hemagglutination Inhibition Test

Preparation of rabbit IgG	100% inhibiting dose, μg	Inhibiting activity, in % of activity of 7S monomer	
		on basis of wt.	on molar basis
7S monomer of IgG	9,7	100	100
F (ab')$_2$ pepsin	100	—	—
F (ab') pepsin	100	—	—
Fab papain	100	—	—
Fc papain	21,6	44	15
Facb plasmin*	89,8	10	8.3
p'Fc pepsin	100	—	—
9,6S dimer of IgG	0,38	2520	1260
7S monomer of IgG Reduced	30,0	32	32

*According to results of RPCAT, plasmin Facb contains 7% undigested IgG as impurity.

To obtain F(ab')$_2$- and pFc'-fragments, the IgG was hydrolyzed with pepsin [10] and the digest was fractionated on a Sephadex G-200 column. The Facb-fragment of IgG was obtained with the aid of plasmin [3].

Activity of protein A was determined by the passive hemagglutination test (PHT) and expressed as its minimal dose causing agglutination of sheep's red blood cells sensitized with a subagglutinating dose of rabbit hemagglutinin IgG.

The ability of the preparations of IgG and its fragments to interact with protein A was estimated by the passive hemagglutination inhibition test (PHIT). To perform the PHIT, different doses of test preparations of IgG or its fragments were kept for 1 h at 20°C with a dose of protein A corresponding to four times its titer in the PHT. Simultaneously with the experimental tests, controls were carried out in which the quantity of protein A (test system) and of IgG and its fragments used in the experiment were determined. At the end of incubation sensitized red cells were added to the experimental and control samples. The reaction was read after incubation for 18 h at 4°C. The ability of IgG and its fragments to interact with protein A was expressed as the quantity of the test preparation (in μg) which caused 100% inhibition of the PHT compared with the test system (100% inhibiting dose).

The immunochemical analysis of IgG and its fragments was carried out by immunoelectrophoresis [1], double diffusion in agar [1], gel chromatography [1], sedimentation analysis, and by the reversed passive cutaneous anaphylaxis (RPCAT) test on guinea pigs [11]. Donkey and goat sera against rabbit IgG and its Fc-fragment were used.

EXPERIMENTAL RESULTS

The preparation of rabbit IgG used was homogeneous for molecular weight; the results of immunoelectrophoresis showed that the F(ab')$_2$- and Fab-fragments were homogeneous in the ultracentrifuge and had sedimentation constants of 5.2 and 3.5 S respectively. The Fc-fragment with a sedimentation constant of 3.5 S gave a reaction of partial identity with IgG and showed complete antigenic nonidentity with the Fab-fragment. The pFc'-fragment during gel-filtration on a column with Sephadex G-200 was eluted with a volume of buffer corresponding to protein with a molecular weight of 20,000 daltons. The Facb-fragment occupied an intermediate position between IgG and F(ab')$_2$ during chromatography on Sephadex G-200. Facb fractions minimally overlapping with the IgG and F(ab')$_2$ peak were selected for investigation. Contamination of the Facb preparation by IgG was assessed quantitatively by the RPCAT, in which donkey serum against rabbit IgG was used for the intravenous reacting injection. Centers responsible for cytophilic activity of IgG are known to be located in its $C_\gamma 3$ domain, which is absent in the Facb-fragment [4]. The minimal sensitizing does of IgG was 2 μg and of Facb

30 μg, in the RPCAT. It was concluded from these results that the Facb preparation contained not more than 7% of undigested IgG as an impurity.

It follows from Table 1 that none of the fragments of the rabbit IgG molecule except Fc interacts with protein A, in agreement with data published previously [14]. The trace activity shown by Facb corresponded to activity of the undigested IgG present as an impurity in the test preparation.

The Facb-fragment is known to contain $C_\gamma 2$ domains of heavy chains, whereas the pFc'-fragment consists of a dimer of $C_\gamma 3$ domains of IgG heavy chains [4, 15]. In Facb and pFc' taken together all domains composing the Fc-fragment are thus represented. However, unlike the latter, neither Facb nor pFc' binds with protein A. It can therefore be assumed that $C_\gamma 2$ and $C_\gamma 3$ domains and(or) the flexible segments of the heavy polypeptide chain connecting them take part in the formation of the binding site for this protein. If this suggestion is correct, destabilization of the structure of the Fc-fragment of the IgG molecule would result in a decrease in its affinity for protein A.

In this connection it is important to note that the ability of the Fc-fragment to bind protein A, expressed on a molar basis, is only one-sixth that of the native monomer (Table 1). Since the $C_\gamma 2$ domains are not in contact with each other [5], the disulfide bond between the heavy chains in the hinge region of the molecule must play an essential role in their spatial orientation. Since this bond is absent in the papain Fc-fragment, the decrease in its affinity for protein A compared with native IgG can be explained by disturbance of the mutual orientation of the $C_\gamma 2$ and $C_\gamma 3$ domains. Other evidence of this is given by the results obtained during testing interaction between protein A and rabbit IgG in the molecule of which the disulfide bond between the heavy chains was reduced. In its ability to bind protein A, reduced IgG was only one-third as active as native IgG.

Data on binding of protein A by IgG dimers also are given in Table 1. These dimers are formed spontaneously, they have a sedimentation constant of 9.6 S, and they can be separated from 7S IgG monomers with a high degree of completeness [9]. On the basis of weight, the binding activity of the dimers is 25 times greater than that of the IgG monomers, and on a molar basis they are 12 times as active as the monomers. Aggregation of IgG takes place [2] by means of the Fc-fragment of the molecule and may lead to spatial screening of the binding sites for protein A and, consequently, to a decrease in the binding activity of the dimers. Since the opposite effect was observed in the present experiments, this suggests that the strengthening of binding of protein A through dimerization can be explained most probably by stabilization of the conformation of the Fc-fragment in the composition of the dimer and, in particular, by the stricter orientation of the $C_\gamma 2$ and $C_\gamma 3$ domains relative to each other.

It can be concluded from these results that not only the presence of a covalent bond between $C_\gamma 2$ and $C_\gamma 3$ domains, but also strictly definite mutual orientation of these domains is essential for protein A binding.

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