IMMUNOLOGICAL PROPERTIES OF PAPAIN-DIGESTED ANTIBACTERIAL AND ANTIRICKETTSIAL RABBIT SERA

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According to recently obtained data [3, 8, 9, 10], papain digestion of γ-globulins from rabbit sera immune to protein and conjugated antigens produces the formation of fragments of the γ-globulin molecule, which lose their precipitating properties, but retain the ability to unite specifically with the antigen; this is reflected in their property of inhibiting the precipitation reaction of the unchanged serum with the corresponding antigen. Physicochemical studies have shown [8, 10] that γ-globulins split by papain separate into three fragments with a sedimentation constant of 3.5 S_{20}. Porter [9, 10] found that when antibodies to pneumococcal polysaccharides are split by papain, they lose their precipitating properties, but, unlike the anti-protein rabbit antibodies, they do not possess a marked ability to inhibit specifically the precipitation reaction. The above indicates either a destruction by papain of anti-polysaccharide antibodies, or certain definite peculiarities in the reaction between papain-split anti-polysaccharide antibodies and their antigen.

Because of this peculiar behavior of papain-split anti-pneumococcal antibodies we considered it of interest to find out what effect papain digestion would have on serological properties of antibodies to other polysaccharide antigens of bacterial origin and to rickettsiae.

METHODS AND RESULTS

In our experiments we have used γ-globulin fractions of rabbit antisera to Vi-coli antigen, complete (O) antigen, as well as γ-globulins against Rickettsia prowazeki. As antigens in our immunological experiments we have used Vi-coli antigen obtained according to A. P. Konikov and V. V. Klyucheva [2], complete (O) antigen of S. typhi obtained according to Westphal [11], and corpuscular antigen of R. prowazeki from lung culture [1] and the rickettsial toxic substance [4].

γ-Globulins were papain-digested according to the method described earlier [3]. Eighteen hours after the beginning of proteolysis, papain was inactivated with monoiodoacetate, and the split γ-globulin was dialyzed at 2°C for 48 hours against distilled water. A precipitate of inactive proteins, formed during dialysis (preliminary experiments), was separated by centrifugation, after which the supernatant fluid was used in immunological experiments.

Papain-split γ-globulins were tested in precipitation, agglutination and complement-fixation reactions. We have also tested the ability of the split globulins to inhibit specifically the precipitation reaction and their protective action in mice.

Results of the experiments are presented in Table 1.

According to the data obtained, papain-digested γ-globulins from antisera to Vi- and O-antigens lose their precipitating and agglutinating properties and do not fix the complement in the presence of the specific antigen, and cannot inhibit the reaction of precipitation between the unchanged antiserum and its antigen. Papain-digested γ-globulin from rabbit antiserum to R. prowazeki also loses its serological properties in vitro.

At the same time, the investigation of the protective properties of the split immune globulins has shown that they retain their ability to protect animals specifically from lethal doses of S. typhi culture and from toxic rickettsial substances. These results show that papain-digested γ-globulins from antibacterial and antirickettsial rabbit sera contain serologically active fragments of the antibody molecule, which retain their ability to unite specifically with the antigen, in spite of the total loss of their ability to react serologically in vitro.

TABLE 1. Immunological Properties of Papain-Digested γ-Globulins from Antisera to Bacterial Polysaccharides and Rickettsiae

Sera tested	Serological reactions				Protective properties (RD^{50} in mg of protein)
	precipitation	agglutination	complement fixation	inhibition of precipitation reaction	
γ-globulin from anti-Vi-coli serum	+	+	+	0	1.5
same γ-globulin, papain-digested	–	–	–	–	11.0
control (non-immune, papain-digested γ-globulin)	–	–	–	–	–
γ-globulin from anti-complete-O-antigen serum	+	+	+	0	0.13
same γ-globulin, papain-digested	–	–	–	–	0.18
control	–	–	–	–	–
γ-globulin from anti-R. prowazeki serum	0	+	+	0	0.11
same γ-globulin, papain-digested	0	–	–	0	0.22
control	0	–	–	0	–

Legend: RD^{50}—see ref. [12];* +)positive reaction; –)negative reaction; 0)reaction not tested.

The presence of active fragments of the antibody in digested anti-polysaccharide rabbit sera was demonstrated in in vitro experiments, using globulin immune to Vi-coli antigen. For this, the papain-digested γ-globulin was specifically adsorbed on the insoluble Vi-coli antigen.

The insoluble Vi-coli antigen was prepared by its precipitation with protamine, taking 2 mg protamine for 1 mg of Vi-coli antigen. Control experiments have shown that Vi-coli antigen prepared by this method fully retains its ability to unite with specific antibodies. The insoluble Vi-coli antigen (immunosorbent) prepared by this method and thrice washed with physiological saline was suspended in digested γ-globulin from an anti-Vi-coli serum. After 2 hours the precipitate was centrifuged down, washed with cold saline, and then protein concentration in it was determined by the method of Folin-Ciocalteu[7]. Papain-digested non-immune γ-globulin was used as control.

TABLE 2. Amount of Protein AFA (in mg) Extracted by the Immunosorbent from Papain-Digested Immune γ-globulin of the Anti-Vi-Coli Rabbit Serum

Experiment	Control (digested non-immune globulin)
0.657	0.108

Note. In the experiment 0.4 mg of Vi-antigen adsorbed on protamine was taken in correspondence to 1 ml of digested γ-globulin.

As shown in Table 2, the immunosorbent extracts from the digested immune γ-globulin considerable amounts of protein, as compared with the control. Since protamine and Vi-antigen do not contain aromatic amino acids, which may react with the Folin reagent, the entire increase of protein in the experimental test is accounted for by the specific adsorption of active fragments of the digested antibody on the immunosorbent. In fact, as it was seen in subsequent experiments, the digested γ-globulin from anti-Vi-coli serum, after its impoverishment on the immunosorbent, loses completely its protective properties in in vivo experiments.

*As in original, although there are only 11 references listed; possibly [2] is meant, or else [11] [Publisher's note].

Thus, the experimental data presented above show that papain-digestion of rabbit γ-globulins against bacterial polysaccharides and rickettsiae produces serologically active fragments of the antibody molecule. From this it follows that there are no significant differences in the structures of anti-protein and anti-polysaccharide antibodies in animals of the same species.

At the same time the question arises, why the papain-fermented anti-polysaccharide rabbit antibodies, unlike anti-protein antibodies, do not exhibit their activity in the reaction of inhibition of precipitation. It may be supposed that this is related to peculiarities of interaction of anti-polysaccharide antibodies with their antigens. It is known that the bonds of polysaccharide antigens with the specific antibodies are weaker than those of protein antigens; this is shown, for example, in the relatively easy dissociation of complexes of polysaccharides and specific antibodies in concentrated solutions of certain salts [5,6]. Consequently, it is possible that in reactions of inhibition of precipitation, using fermented antibodies to bacterial polysaccharides, the addition of native antiserum leads to the expulsion of the active fragment of antibody from its complex with the antigen, thus preventing the reaction of inhibition of precipitation.

SUMMARY

In papain digestion of immune γ-globulin from rabbit sera against bacterial polysaccharides and rickettsia, antibody molecular fragments are formed, producing no reactions of precipitation, agglutination, or complement fixation. These fragments are also incapable of specific inhibition of the precipitation reaction of unchanged antiserum. At the same time the products formed from antibody splitting are capable of specific interaction with the antigen; this was confirmed in vivo in experiments (protection test), as well as in vitro with the specific immunosorbent.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
