THE STUDY OF THE SEROLOGICAL ACTIVITY OF PAPAIN-DEGRADED ANTIBODIES BY MEANS OF THE COMPLEMENT FIXATION REACTION

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The monovalent fragments of rabbit antiprotein antibodies obtained as a result of proteolytic degradation with papain [1,2] do not give a precipitation reaction. The ability of these fragments to combine with a specific antigen may be detected, as Porter has shown, by the precipitation delaying reaction [1].

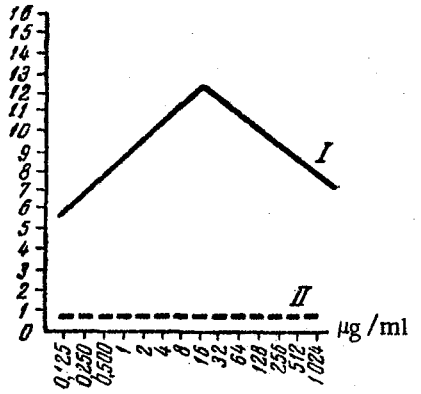

Fig. 1. Curves of fixation of complement (C^1) in the systems: I) crystalline human serum albumin – eluate of specific rabbit antibodies (0.21 mg protein/1 ml); II) crystalline human serum albumin – eluate of active fragments of rabbit antibodies (0.021 mg protein/1 ml).

We have investigated the behavior of active fragments of antibodies (AFA), isolated from antiserum degraded with papain [3], by means of a more sensitive serological test, namely, the complement fixation reaction, capable of demonstrating much smaller concentrations of antibody or antigen than the precipitation reaction [4,5,6]. The high sensitivity of the complement fixation reaction enables the investigation to be carried out with small amounts of test material, which is particularly important in the study of AFA, the production of which, in large amounts, is beset by considerable difficulties.

EXPERIMENTAL METHOD

We used crystalline human serum albumin, obtained by Adair and Taylor's [7] method, crystalline horne serum albumin, obtained by Adair and Robinson's [6] method, and rabbit sera against these antigens for our experiments. The gamma-globulins from the immune rabbit sera were obtained by salting out with ammonium sulfate at 34% saturation. Antibodies were isolated from the immune gamma-globulin by the method of specific serological adsorption by means of antigen fixed on an insoluble carrier, by Campbell's method [8].

The gamma-globulins were hydrolyzed with papain by Porter's method [12]. After inactivation of the enzyme with monoiodoacetate, the hydrolyzed gamma-globulin was dialyzed against distilled water at 2 ° for 48 h. The precipitate of inactive proteins thrown down during dialysis [10] was discarded, and the supernatant fluid (the P-fraction), containing the active fragments of the antibody molecule [10], was used in the subsequent experiments. Besides active fragments of antibodies, the P-fraction also contained hydrolyzed nonimmune globulin.

Active antibody fragments were isolated in a pure form from papain-digested immune gamma-globulin by the method of specific serological adsorption, using antigen fixed to an insoluble carrier [3,10].

The precipitation reaction was performed in Heidelberger and Kendall's quantitative modification. The precipitation delaying reaction was carried out by Porter's method [12]. A quantitative modification of the complement fixation reaction was used [1,5]. Protein was determined by the Folin-Ciocalte method.

EXPERIMENTAL RESULTS

The study of the activity of the P-fraction and of the pure AFA in the complement fixation reaction showed that neither will fix complement in the presence of the specific antigen. Meanwhile, the nonhydrolyzed antibodies

TABLE 1. Delay in the Complement (C^1) Fixation Reaction at the Point of Equivalence of the Test System and Different Doses of P-Fraction

	Tube No.	P-frac. (ml)	Volume of						Titration of C^1			
			antigen (in ml) (3.6 μg/ml)	physiol. saline (ml)		C^1 (1:3) (ml)	anti-serum (1:200) (ml)		dose taken at titra. (ml)	% hemol.	Amount of C^1 (50% hemolytic units)	
											free	fixed
Expt.	1	1.18	0.25	–	Incubation at	0.1	0.25	Incubation in the	0.07	35	7.6	7.5 – 7.6 = 0
	2	0.56	0.25	–	room temp.	0.1	0.25	cold for 14 h	0.07	40	8.1	8.1 – 8.1 = 0
	3	0.28	0.25	–	for 1 h	0.1	0.25		0.07	40	8.1	8.1 – 8.1 = 0
	4	0.14	0.25	–		0.1	0.25		0.1	30	4.8	8.1 – 4.8 = 3.3
	5	0.07	0.25	–		0.1	0.25		0.3	70	2.5	8.1 – 2.5 = 5.6
	6	0.035	0.25	–		0.1	0.25		0.5	60	1.32	8.1 – 1.32 = 6.8
Test system	7	–	0.25	–		0.1	0.25		0.4	45	1.4	8.1 – 1.4 = 6.7
Control	8	–	0.25	0.25		0.1	–		0.05	25	9	
	9	–	–	0.25		0.1	0.25		0.07	40	8.1	
	10	1.18	0.25	–		0.1	–		0.1	70	7.5	
	11	0.56	0.25	–		0.1	–		0.07	40	8.1	
	12	–	–	0.5		0.1	–		0.05	25	9	

TABLE 2. Determination of the Point of Equivalence of the Test System in the Precipitation Reaction in Terms of the Protein Content of the Precipitate

	Antigen, 0.2 ml (mg/ml)							
Antiserum (1 : 2), 0.2 ml	4	2	1	0.5	0.25	0.12	0.06	0.03
	Protein content of precipitate (in mg)							
	–	Traces	0.04	0.324	0.410	0.12	0.07	Traces

TABLE 3. Delay of Precipitation Reaction at the Point of Equivalence of the Test System with Different Doses of P-Fraction

Quantity of					
P-fraction (in mg)	Antigen (0.25 mg/ml) (in ml)		Antiserum (1 : 2) (in ml)		Protein in precipitate (in mg)
23.2	0.2	Incubation at room	0.2	Incubation in the cold	–
11.6	0.2	temperature for 1 h	0.2	for 24 h	0.197
5.8	0.2		0.2		0.396
2.9	0.2		0.2		0.410
–	0.2		0.2		0.408

obtained from the immune adsorbent from the same sera, equal in their protein content to AFA, possessed high complement-fixing activity. As an example, we show the curves of complement fixation in the system: human serum – albumin – active fragments of antibodies (Fig. 1).

Since AFA had the power of combining with antigen to give a precipitation delaying reaction, it seemed very likely that, although they had not the power of fixing complement, their serological activity might be revealed by their effect in delaying the fixation of complement.

In experiments to study the delay in fixation of complement, we used the P-fraction from rabbit immune gamma-globulin against horse serum albumin. The corresponding test system consisted of crystalline horse serum albumin and a rabbit antiserum to this antigen.

The complement-fixation delaying reaction was performed with optimal proportions of antigen and antibody in the test serum, giving maximal fixation of complement. The discovered optimal amount of antigen was incubated with different amounts of P-fraction for 1 h at room temperature. At the end of incubation complement was added to the mixture of P-fraction and antigen, and the optimal amount of antiserum determined in preliminary experiments was also added. Controls of fixation of complement by the test system, and of the P-fraction, the antigen, and the antiserum for anticomplement activity were carried out at the same time. The experimental and control tubes were placed in a refrigerator overnight. The amount of fixed complement was determined in the usual manner [13], in 50% hemolytic units, by the back-titration method.

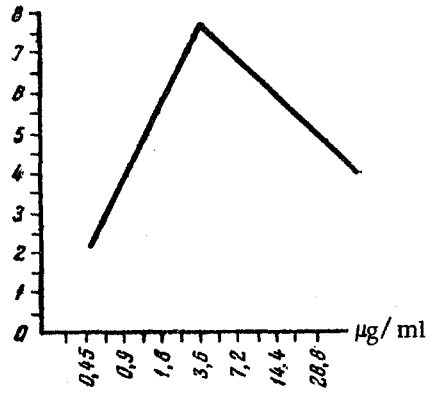

Fig. 2. Curve of fixation of complement (C^1) in the test system (crystalline horse serum albumin – rabbit antiserum in a dilution of 1 : 200).

The results of the titration of the test system for determining the optimal proportions of antigen and antiserum are shown in Fig. 2. With serum in a dilution of 1 : 200, maximal fixation of complement was observed with an antigen concentration of 3.6 μg crystalline serum albumin in 1 ml.

The results of the experiments to study the delay in complement fixation by the P-fraction with selected concentrations of antigen and serum are shown in Table 1, from which it is clear that the P-fraction did cause delay in the complement fixation reaction, and also that the degree of delay was determined by the dose of P-fraction added.

In order to evaluate the complement-fixation delaying reaction quantitatively, the results were expressed in units of 50% delay of fixation. The unit of 50% delay of fixation was taken to be the dose of P-fraction delaying the complement fixation reaction by 50% compared with the fixation in the control test system. It will be clear from Table 1 that a delay of fixation by 50% took place when 0.14 mg of P-fraction was added to the system.

To assess the sensitivity of the complement-fixation delaying reaction, the same preparation of P-fraction was investigated experimentally by Porter's precipitation delaying reaction [1]. The point of equivalence of the test system in the precipitation reaction was determined by Heidelberger and Kendall's method [11] (Table 2). The precipitation delaying reaction was carried out with different doses of P-fraction and with antigen and antiserum in the proportion corresponding to equivalence of the test serum. The figures in Table 3 show that a delay of 50% in the precipitation reaction took place after the addition of 11.6 mg of P-fraction.

It is thus possible, by means of the complement-fixation delaying reaction, to determine amounts of P-fraction 80 times smaller than by means of the precipitation delaying reaction (11.6 : 0.14).

The method of carrying out the complement-fixation delaying reaction developed on the P-fraction was used to titrate pure AFA and also the products of their further hydrolysis. Preliminary experiments showed that products of further hydrolysis of AFA with papain possesses extremely low activity in the precipitation delaying reaction, although they gave a quite definite delay of the complement fixation reaction. It is probable that the method of the complement-fixation delaying reaction, as described above, may be used widely in experimental immunology, more particularly for the detection of incomplete antibodies.

SUMMARY

As demonstrated, products of papain degradation of immune gamma-globulin do not fixate the complement in the presence of specific antigen; however, they competitionally inhibit the complement fixation reaction by unchanged serum and specific antigen. To ascertain the serological activity of papain-degradated antibodies, a method of inhibiting the complement fixation reaction has been developed, the sensitivity of which is 80-100 times that of the method of precipitation reaction inhibition used for this purpose formerly.

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