ISOLATION OF THE CENTER OF SPECIFIC ACTIVITY OF AN ANTIBODY FROM AN ANTISERUM HYDROLYZED WITH PAPAIN, USING AN ANTIGEN FIXED ON CELLULOSE

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(Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 76-79, November, 1960
Original article submitted October 10, 1959

It has previously been shown [4-7] that, as a result of the action of the proteolytic enzyme papain on an immune globulin, the molecule of the latter is split up into several parts, without loss of the power of this γ-globulin to react specifically with its own antigen.

All attempts to isolate from the products of proteolysis in a pure form the fragment of the globulin molecule possessing the specific antideterminant, using chemical or physicochemical methods [6, 7] for this purpose, have, however, been unsuccessful.

It accordingly appeared to be of great interest to use the specific antigen– antibody reaction to isolate the active center of the antibody from the products of proteolysis, using for this purpose an antigen fixed on an insoluble carrier (cellulose).

EXPERIMENTAL METHOD AND RESULTS

The immune sorbent in our experiments was horse albumin, fixed on cellulose by Campbell's method [3], which we modified.*

A rabbit immune serum against crystalline horse albumin was fermented with papain** for 16 hours at 37°. The papain was preliminarily activated by being allowed to stand in a buffer solution at pH 7.0, consisting of equal parts of a 0.05M solution of cystein hydrochloride, a 0.01M solution of Na-versene and a 0.3M solution of K_2HPO_4. The duration of activation was 1 hour at 42°. The quantity of papain added to the serum was 0.5 mg for each 70 mg of serum protein.

After the completion of the fermentation of the serum, it was cooled to 2°, to it was added a 0.05M solution of monoiodoacetate (papain inhibitor), and it was then subjected to rapid dialysis in the cold against physiological saline. Dialysis continued for 28-30 hours.

The experiments showed that a serum, when fermented in this way, completely lost its precipitating properties, but at the same time preserved its power of specific combination with its antigen (horse albumin), which could be judged by the ability of this serum to specifically inhibit the precipitation reaction between horse albumin and unchanged serum. In subsequent work this test has been used for detection of the specific antideterminant in a fermented serum.

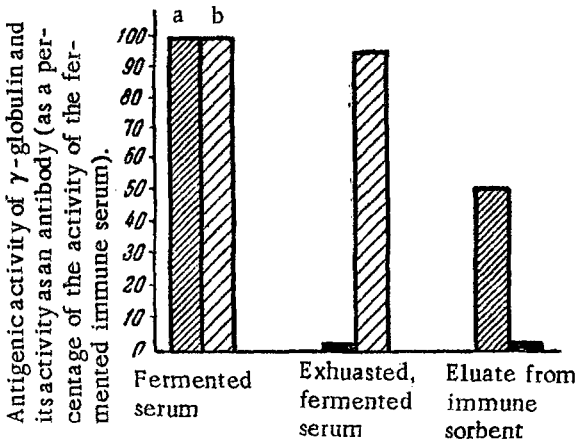
* In contrast to Campbell, the cellular tissue was not combined with p-nitrobenzylchloride but with p-nitrobenzylbromide, which has a much higher reactive power.

** The preparation of papain was obtained from the Baker Research Laboratory, London.

Protein Content of Specific Precipitate (in mg) Obtained as a Result of the Reaction between a Fermented Rabbit Immune Serum (Antigen) and an Ass's Serum Against Rabbit Globulin

Antibody	Quantity of antigen (in mg)							
	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64
	quantity of protein in specific precipitate (in mg)							
Fermented serum	0,223	0,344	0,429	0,671	0,809	0,945	1,083	0,573
Fermented serum, exhausted with immune sorbent	0,172	0,297	0,395	0,621	0,730	0,890	0,985	0,521
Eluate from immune sorbent	—	—	—	—	—	—	—	—

Note. In the experiments 0.25 ml of antiserum, diluted 1 : 2, and a corresponding amount of antigen were used.



The presence of the antigenic determinant of the γ-globulins (b) and the antideterminant of the antibody (a) in an immune serum after fermentation with papain, in the same serum when exhausted with immune sorbent, and in the eluate from the immune sorbent.

In order to isolate the specifically active center of the antibodies, the immune serum, after fermentation as described above, was mixed with the immune sorbent for 2 hours at room temperature, after which the supernantant fluid was tested for its content of antideterminant in inhibition experiments. These experiments showed that the immune sorbent completely extracted from the serum the fragment of the γ-globulin molecule possessing specific antibody activity, as a result of which the serum, after exhaustion by the immune sorbent, lost its ability to specifically inhibit the precipitation reaction.

After the thorough removal of proteins not reacting with the immune sorbent, by means of the repeated washing with physiological saline on a Büchner funnel, elution of the specific inhibiting factor from the immune sorbent was carried out. The elution was done by acidification to pH 2.6 for 2 hours at room temperature, after which the presence of specifically active centers of the antibody was determined in the eluate.

The experiments showed that the eluate does, in fact, contain the antideterminants of the antibodies, as disclosed by its ability to specifically inhibit the precipitation reaction between the unchanged serum and its antigen.

Determination of the protein content in the eluate showed that, taking account of all the volume changes, it comprised one third of the total protein content of the antibodies in the test serum (8.6 mg antibody protein

in 1 ml of serum compared with 3.22 mg protein in 1 ml of eluate). Under these circumstances the eluate preserved about one half the inhibiting activity of the fermented serum.

In connection with these findings it was thought to be most important to discover whether the fragment of the antibody molecule which we had isolated, bearing the specific antideterminant, possessed the antigenic activity appertaining to rabbit globulin.

Experiments showed that a rabbit serum against horse albumin, subjected to fermentation and used as antigen, gave a precipitation reaction with the immune serum of an ass against rabbit globulin. After exhaustion of the fermented serum with the immune sorbent, which led to the loss of its function as an antibody, it nevertheless fully retained its ability to precipitate an ass's antiserum. Meanwhile the eluate from the immune sorbent not only did not precipitate the ass's antiserum, but also it did not inhibit the reaction of the latter with unchanged rabbit globulin (see Table).

The results obtained (see Figure) show that the specifically active center of the antibody and the antigenic determinant inherent in γ-globulin are found in different parts of the antibody molecule, which enables the specific antideterminant to be isolated in a pure form, not possessing the antigenicity of γ-globulin.

As Porter [6] reported recently, during fractionation of immune globulin, fermented with papain, on a chromatographic column, he was able to obtain three fractions, two of which contained active centers of the antibody in addition to inactive protein, and one possessed only the properties of an antigen. These results are in complete agreement with our own.

According to the findings of Treffers [8] and Wright [9], and as a result of systematic investigations carried out recently by G. N. Kryzhanovskii, L. N. Fontalin and L. A. Pevnitskii [1, 2], it has been shown that antibodies do not possess antigenic properties which differ from the antigenic properties of γ-globulin. Our findings, according to which the fragment of the antibody molecule bearing the antideterminant does not possess the inherent antigenic properties of γ-globulin, support the above investigations to a certain degree.

SUMMARY

A cellulose-fixed antigen was used to isolate the specifically active antibody center from papain-fermented antiserum. This antibody center possessed no antigenic properties characteristic of γ-globulin.

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