ISOLATION OF Fab AND Fc FRAGMENTS FROM MOUSE IMMUNOGLOBULIN G1

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Two methods of obtaining Fab and Fc fragments from mouse immunoglobulin G1 are described. In the first case the papain protein digest is fractionated on a column with DEAE- or DE-32-cellulose, equilibrated with 0.005 M potassium phosphate buffer, pH 8.0. The Fab fragment is eluted from the column in the starting buffer; the fragment is eluted when the ionic strength of the buffer is increased to 0.4 M. In the second case the protein is fractionated on an ion-exchange resin equilibrated with 0.004 M Tris-H_3PO_4 buffer, pH 8.5. The whole of the protein applied under these circumstances is bound by the column. The Fab fragment is eluted with 0.04 M Tris buffer containing 0.004 M of a mixture of K-phosphate salts at pH 8.5; the Fc fragment is eluted by increasing the ionic strength by means of phosphate to 0.4 M. Since neither method can yield absolutely pure Fab or Fc fragments, in order to obtain monospecific antisera against these fragments it is necessary to cross-exhaust the antisera with appropriate immunosorbents.

KEY WORDS: immunoglobulin; Fab fragment; Fc fragment; ion-exchange chromatography.

For various purposes pure fragments of immunoglobulins are needed. The method of fractionating rabbit immunoglobulin into Fab and Fc fragments was suggested as long ago as in 1959 [5]. However, the isolation of these fragments from immunoglobulins of other species of animals, especially mice, is still difficult. For instance, although methods of isolating Fab and Fc fragments from mouse myeloma protein, belonging to the IgG2a subclass, and of mouse serum immunoglobulins have been described [2, 4], no method has yet been developed for isolating these fragments from mouse immunoglobulin of the G1 class (IgG1).

Accordingly an attempt was made to develop methods of isolating Fab and Fc fragments from mouse IgG1.

Immunoglobulins isolated from the serum of BALB/c mice inoculated subcutaneously with an MOPC 21 plasmacytoma (the authors are grateful to Dr. M. Potter fro providing the tumor) were used as IgG1. The IgG1 were isolated by precipitation with 40% $(NH_4)_2SO_4$ and purified on a column with DEAE-cellulose [6].

The IgG1 thus isolated was subjected to restricted papain hydrolysis by the method in [3]. The protein (10-15 mg/ml) was dissolved in 0.075 M Na phosphate buffer, pH 7.0, containing 0.075 M NaCl, 0.01 M cysteine, and 0.002 M EDTA, papain (BDH, twice crystallized) was added in the ratio 1:100, and the mixture was incubated for 2 h at 37°C. The digest was dialyzed at 4°C, first against distilled water for 48 h and then against the buffer used for ion-exchange chromatography.

The fragments were separated on DEAE- or DE-32 cellulose columns in two buffer systems: 0.005-0.5 M K phosphate buffer, pH 8.0, or in Tris-H_3PO_4 buffer, pH 8.5, containing K phosphate salts.

The product of restricted papain hydrolysis were investigated by sedimentation analysis (for which the authors are grateful to Dr. V. O. Shpikiter and Dr. I. M. Karmanskii) and by immunoelectrophoresis in agar gel [1].

Antisurum against IgG1 obtained from rabbits immunized with IgG1 in Freund's complete adjuvant (Difco) by injection into the popliteal lymph nodes and reimmunized at three points of

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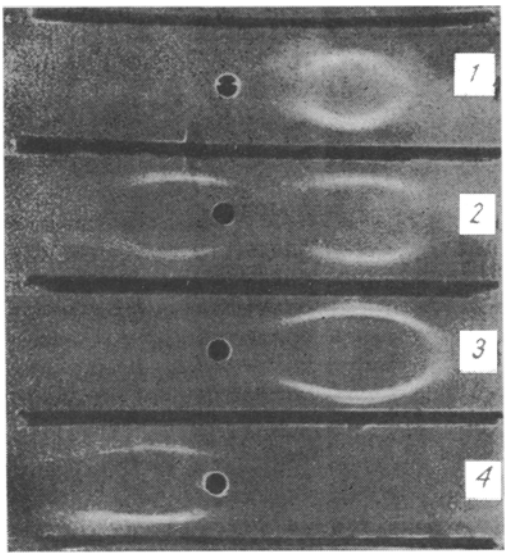

Fig. 1

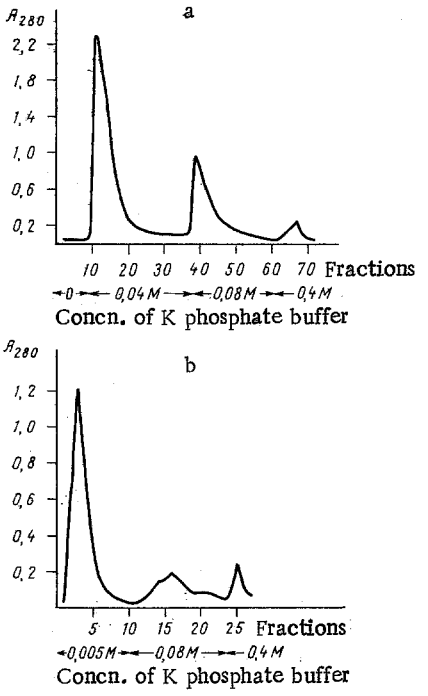

Fig. 2

Fig. 1. Distribution of IgG1, papain hydrolysis products of IgG1, and Fab and Fc fragments isolated from digest during immunoelectrophoresis in agar gel. 1) IgG1; 2) papain digest of IgG1; 3) Fab fragments; 4) Fc fragments. Gutters contain rabbit antiserum against mouse IgG1.

Fig. 2. Chromatographic fractionation of Fab and Fc fragments of mouse IgG1 on column with DEAE-cellulose equilibrated with 0.005 M K phosphate buffer, pH 8.0 (a), and on column with DEAE-(De-32) cellulose, equilibrated with 0.004 M Tris-H_3PO_4-buffer, pH 8.6 (b). Abscissa, molarity of K phosphate; ordinate, absorption at 280 nm (A_{280}).

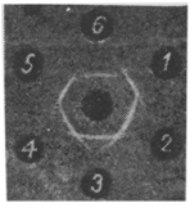

Fig. 3. Immunodiffusion of IgG1, papain digest, and Fab and Fc fragments isolated from it in agar gel. Central well contains rabbit antiserum aganist mouse IgG1. 1) IgG1; 2, 4) Fab fragemnt; 3, 6) Fc fragments; 5) papain digest.

the body one month later. Serum was taken one week after reimmunization on alternate days for two weeks.

The sedimentation coefficient of the papain fragments of IgG1 was 3.5S. The results of immunoelectrophoresis are shown in Fig. 1. Most of the protein in the digest was found to consist of IgG1 fragments, although there was also a very small quantity of undigested IgG1. The Fc fragment, with greater electrophoretic mobility, migrates toward the anode, whereas the Fab fragment, like IgG1 (and more strongly still), is carried by electroendoosmosis toward the cathode.

On application of the digest to the column with the ion-exchange resin (20-50 mg protein/g cellulose), equilibrated with 0.005 M K phosphate buffer, pH 8.0, most of the protein was eluted from the column in the same buffer (ran through). A smaller proportion binds with the column under these conditions and can be eluted by an increase in the ionic strength of the buffer. Some of the bound protein is eluted by an increase in ionic strength to 0.04 M, but some only by an increase to 0.4 M buffer (Fig. 2a).

On application of the digest to the column equilibrated with 0.004 M Tris-H_3PO_4 buffer, pH 8.5, practically all the applied proteins is bound by the ion-exchange resin. Stepwise elution (after washing the column with the starting buffer) with solutions of 0.004 M Tris buffer, pH 8.5, containing 0.04, 0.08, and 0.4 M mixtures respectively of K phosphate salts, pH 8.5, gives a distribution of protein shown in Fig. 2b.

Analysis of the fractions obtained by the two methods of elution by double immunodiffusion and immunoelectrophoresis showed that the protein eluted together with the starting buffer in the first case or in peak 1 in the second case differs in its antigenic properties from the protein eluted after an increase in the ionic strength (Fig. 3), although they were partly identical with the original IgG1 (and the papain digest of IgG1). The fractions which did not bind or which bound less firmly with the ion-exchange resin during immunoelectrophoresis possessed the mobility of the Fab fragment, whereas fractions eluted after an increase in ionic strength possessed the mobility of the Fc fragment respectively (Fig. 1).

The ratio of Fab to Fc fragments in different experiments varied from 2:1 to 5:1; the total yield of protein from the column did not exceed 80% (deviations of the ratio between the fragments from the theoretically possible ratio may be due, first, to the presence of certain quantities of hemoglobin in the IgG1 preparation and, second, to the greater ease of digestion of the Fc than of the Fab fragments under these experimental conditions).

Concentration of the preparation revealed slight contamination with intact IgG1. That was probably why antisera obtained by immunization of rabbits with the fragments in Freund's complete adjuvant proved not to be monospecific. It was impossible to purify the Fab and Fc fragments further by gel-filtration on a column with Sephadex G-150. Accordingly, in order to obtain monospecific antisera against Fab and Fc fragments of mouse IgG1, cross-exhaustion is necessary with the appropriate immunosorbents.

The use of the two methods of deposition and elution of papain fragments of mouse IgG1 on columns with DEAE- or DE-32 cellulose showed that the degree of purity of the fragments thus obtained was virtually the same. Because of its greater simplicity, preference must obviously be given to the first method.

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