Journal of Chromatography, 189 (1980) 428–432

CHROM. 12,467

Note

Simple method for the purification of C1q, a subcomponent of the first component of complement by affinity chromatography using IgG-Sepharose

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(Received August 20th, 1979)

The serum protein that is the first component of complement is a macromolecular complex composed of three subcomponents, C1q, C1r and C1s*, which are complexed with each other in the presence of calcium ion[1]. C1q has been purified from human serum by repeated precipitation at low ionic strength and dissolution in salt solution[2] or by precipitation at low ionic strength, gel filtration and ion-exchange chromatography[3]. Assimeh *et al.*[4], on the other hand, attempted to isolate the subcomponents of C1 by affinity chromatography using IgG-Sepharose. Rabbit C1q was also purified from the euglobulin fraction of serum[3,5].

In order to investigate the relation of the molecular structure of the subcomponents of C1 to their functions, such as binding of C1 to an immune complex, activation of C1 to $C\bar{1}$ and mutual combination of the subcomponents of C1, a simple method for obtaining a highly purified preparation of C1q is desirable. This paper describes the purification of C1q by affinity chromatography on IgG-Sepharose 6B and column chromatography on CM-Sephadex C-50, and the interaction of C1q with immunoglobulin G is discussed.

EXPERIMENTAL

Materials

Sepharose 6B and CM-Sephadex C-50 were purchased from Pharmacia (Uppsala, Sweden). AGLME was a product of the Protein Research Foundation, Osaka, Japan. The proteins used as standards for the measurement of the molecular weights of the subcomponents of C1q were purchased from Behringer (Mannheim, G.F.R.) and Sigma (St. Louis, Mo., U.S.A.).

Procedure

IgG-Sepharose 6B was prepared according to the method of Assimeh *et al.*[4] as described previously[6]. The esterase activity of $C\bar{1}s$ was determined as described in previous paper with the use of AGLME as a substrate[6]. Polyacrylamide gel electro-

* Abbreviations: C1 = first component of complement; AGLME = acetylglycyl-L-lysine methyl ester; SDS = sodium dodecyl sulphate; DTT = dithiothreitol.

phoresis in the presence of SDS was performed according to the method of Weber and Osborn[7]. SDS gel electrophoresis in the presence of urea and that after reduction of C1q with DTT were performed according to the method of Fairbanks *et al.*[8] as described by Reid and Porter[9]. Haemagglutination activities of rabbit C1q were measured with the use of sheep erythrocytes sensitized with a small amount of rabbit IgG haemagglutinin.

RESULTS

Freshly drawn rabbit serum (320 ml) was diluted with an equal volume of distilled water, fractionated by addition of 32 ml of 50% (w/v) polyethylene glycol 4000 and stirred for 30 min at 4°. The resulting precipitates were centrifuged, dissolved in 20 m*M* Tris–hydrochloric and buffer (pH 8.5) containing 0.15 *M* sodium chloride and 5 m*M* calcium chloride and applied to an IgG-Sepharose column (10 × 2 cm) as described previously[6]. After washing the column with the same buffer, $C\bar{1}r$ and $C\bar{1}s$ were eluted with 5 m*M* EDTA containing buffered saline (pH 8.5). In order to eiiminate the protein bound non-specifically on the column, the column was washed with 1.0 *M* sodium chloride solution containing the same buffer and then C1q was eluted from the column with 30% ethylene glycol containing 1.0 *M* sodium chloride and the same buffer (Fig. 1). C1q was detected by the Ouchterlony test using anti-rabbit C1q guinea pig antiserum. In this step, the haemagglutination activity could not be measured because of the inhibitory effect of the elution solvent on the activity.

The pooled C1q fractions were dialysed against 0.01 *M* acetate buffer (pH 5.5) supplemented with 0.15 *M* sodium chloride and 0.01 *M* EDTA and applied to a column of CM-Sephadex C-50 (5 × 1.5 cm) equilibrated with the same buffer. After washing the column with the same buffer, C1q was eluted from the column with a

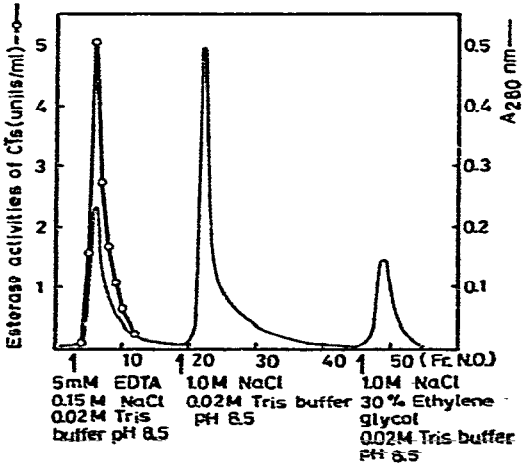

Fig. 1. Isolation of C1q by IgC-Sepharose column chromatography. Fractions of 5 ml (1–18), 10 ml (19–45) and 20 ml (46–56) were collected and their protein contents were measured from their absorbances at 280 nm. The esterase activity of C1s was determined by the hydroxamate method using AGLME as a substrate as described previously[6]. Fractions of C1q were monitored by the Ouchterlony test and pooled.

linearly increasing concentration of sodium chloride. As can be seen in Fig. 2, C1q was eluted from the column as a single protein peak. The haemagglutination activity of C1q was located in this fraction.

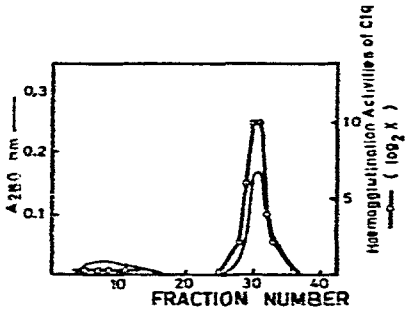

Fig. 2. Isolation of C1q by CM-Sephadex C-50 column chromatography. Fractions of 4 ml were collected and monitored for protein at 280 nm and for haemagglutination activity of C1q.

Rabbit C1q purified by this method formed an immune precipitate band with guinea pig anti-rabbit C1q antiserum in 1% agarose gel containing 1.0 *M* sodium chloride (Fig. 3). When purified rabbit C1q was electrophoresed on 4% polyacrylamide gel in the presence of 0.2% SDS, a single protein band was observed, the molecular weight of which was estimated to be 400,000, relative to the mobilities of the monomer and oligomers of bovine serum albumin (Fig. 4a). This value was consistent with that of human C1q estimated by Yonemasu and Stroud[10].

It is known that human and rabbit C1q are composed of covalently and non-covalently bound subunits[9,10]. To demonstrate the subunit structure, the purified rabbit C1q was treated with urea in the presence or absence of DTT and electrophoresed in the presence of SDS. Purified C1q was dialysed against 4 *M* urea con-

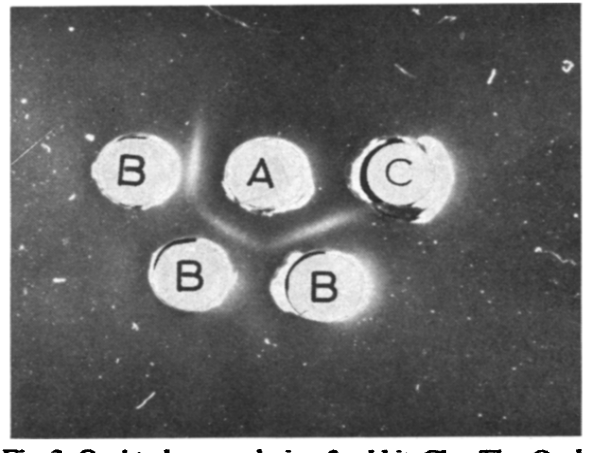

Fig. 3. Ouchterlony analysis of rabbit C1q. The Ouchterlony test was performed on 1% agarose gel containing 1.0 *M* NaCl. Anti-rabbit C1q antiserum was obtained by immunization of guinea pigs with rabbit C1q purified by the method of Volanakis and Stroud[5] and electrophoresed on SDS gel. A, Guinea pig antiserum; B, purified C1q; C, saline.

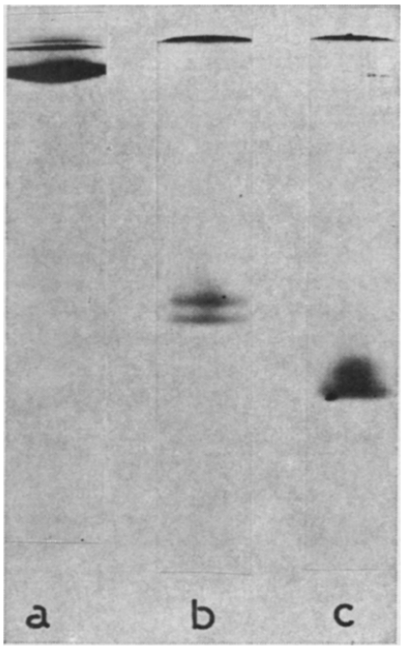

Fig. 4. SDS–polyacrylamide gel electrophoresis of rabbit Clq and its fragments. (a) Clq was electrophoresed on 4% polyacrylamide gel containing 0.2% SDS and 0.2 *M* phosphate buffer (pH 7.2) by the method of Weber and Osborn[7]. (b) Non-covalent subunit of Clq. Clq was incubated with 4 *M* urea containing 1% SDS and 20 m*M* iodoacetamide in Tris–acetate buffer (pH 7.4) for 60 min at 37° as described by Reid and Porter[9]. Electrophoresis was performed according to the method of Fairbanks *et al.*[8] on 6% polyacrylamide gel containing 1% SDS and 0.5% urea in Tris–acetate buffer (pH 7.4). (c) Covalent subunits of Clq. Clq was incubated in 40 m*M* DTT containing 1% SDS, 4 *M* urea and Tris–HCl buffer (pH 7.4) for 60 min at 37° in a nitrogen atmosphere. Alkylation was performed with a 2-fold molar exess of iodoacetamide for 15 min at 37°. Conditions of electrophoresis as in (b).

taining 10 m*M* iodoacetamide and electrophoresed on 6% polyacrylamide gel containing 1% SDS. As can be seen in Fig. 4b, two protein bands were observed, which corresponded to the A–B and C–C dimer chains of Clq reported by Reid and Porter[9]. When Clq was reduced with DTT in the presence of 4 *M* urea and alkylated with iodoacetamide, it was dissociated into smaller subfragments (Fig. 4c), which corresponded to the three covalently linked subunits of human Clq reported by Reid and Poster[9] and Yonemasu and Stroud[10].

With the use of a value of 6.8 for $A^{1\%}_{1cm}$ at 280 nm (ref. 11), 44 mg of Clq was obtained from 320 ml of rabbit serum.

DISCUSSION

Using this simple method for the purification of rabbit Clq by affinity chromatography using IgG-Sepharose and ion-exchange column chromatography, it was found that rabbit Clq was capable of binding with IgG antibodies bound to sheep erythrocytes and of forming immune precipitates against anti-rabbit Clq guinea pig antiserum in agarose gel. The Clq obtained was composed of covalently and non-

covalently bound subunits as in human C1q described by Reid and Poster[9] and Yonemasu and Stroud[10]. Previously, Assimeh *et al.*[4] reported the isolation of human C1q using IgG-Sepharose and observed that C1q bound to IgG-Sepharose was eluted from the column with diaminobutane containing EDTA and sodium chloride. In our work, on the other hand, diaminobutane was found not to be a suitable reagent for the dissociation of rabbit C1q from an IgG-Sepharose column, although $\overline{C1r}$ and $\overline{C1s}$ were eluted from the column. C1q bound to IgG-Sepharose was eluted from the column with 30% ethylene glycol supplemented with 1.0 *M* sodium chloride, but was not eluted by a solution containing only one of these reagents. It is known that ethylene glycol is a useful reagent for the dissociation of the bound protein from a a hydrophobic interaction column[12,13]. These results indicated that both ionic and hydrophobic interactions are involved in the binding of C1q with immunoglobulin G. C1q is able to complex with IgG or IgM, and with $\overline{C1r}$ in the presence of calcium ion[14–16]. The nature of the mutual interactions of the subcomponents of C1 and that between IgG and C1q remain to be clarified.

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