THE EIGHTH COMPONENT OF HUMAN COMPLEMENT (C8): ISOLATION, CHARACTERIZATION, AND HEMOLYTIC EFFICIENCY*‡

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The complement protein C8 plays a key role in the production of membrane damage by the complement system. Whereas cells which have reacted with seven components of complement (C1–C7) are apparently intact with respect to membrane function, these cells undergo lysis after reaction with C8 (1, 2). Cytolysis induced by C8 is independent of C9 and in absence of C9 proceeds at a slow rate, which, however, is greatly accelerated upon addition of the terminal component (3).[1] Accordingly, C8 may be regarded as that protein of complement which is directly responsible for production of complement-dependent membrane damage.

The purpose of the present paper is three-fold: (*a*) A method will be described which permits isolation of C8, a trace protein of human serum, in highly purified and hemolytically active form. (*b*) The isolated protein will be demonstrated as a heretofore unrecognized, immunochemically distinct serum constituent and its molecular properties will be delineated. (*c*) Data will be presented showing that under optimal experimental conditions a few molecules of isolated C8 suffice to cause lysis of a cell.

Materials and Methods

Serum and Purified Complement Components.—Serum was obtained either from fresh or outdated (1 month old) human blood which was purchased from the San Diego Blood Bank. Clotting was induced by addition of $CaCl_2$ and incubation at 37° C for 2–3 hr, after which the blood was held at 4° C overnight. The serum was then separated by filtration through nylon gauze applying manual pressure to obtain maximal yield. C5 (4) and C9 (5) were prepared as described.

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[1] Manni, J. A., and H. J. Müller-Eberhard. 1969. Studies of the mode of action of the eighth component of human complement in immune hemolysis. Manuscript in preparation.

C8 Activity Assay.—Veronal-buffered saline, pH 7.4, containing Ca^{++} and Mg^{++} and 0.1% gelatin (GVB), was used as the diluent in all hemolytic assays (6). The intermediate complex EAC1, 4, 2, 3, 5, 6, 7 (EAC1–7) was prepared as follows.[2] Sheep erythrocytes (E) sensitized with rabbit anti-sheep erythrocyte antibody (A) and carrying the first three components of complement, EAC1, 4, 2, were prepared as described previously (7) using oxidized C2 (8). This intermediate complex was converted to EAC1–7 using a chromatographically obtained reagent containing C3, C5, C6, and C7.

60 ml of human serum was dialyzed against phosphate buffer, pH 6, ionic strength 0.1, for 24

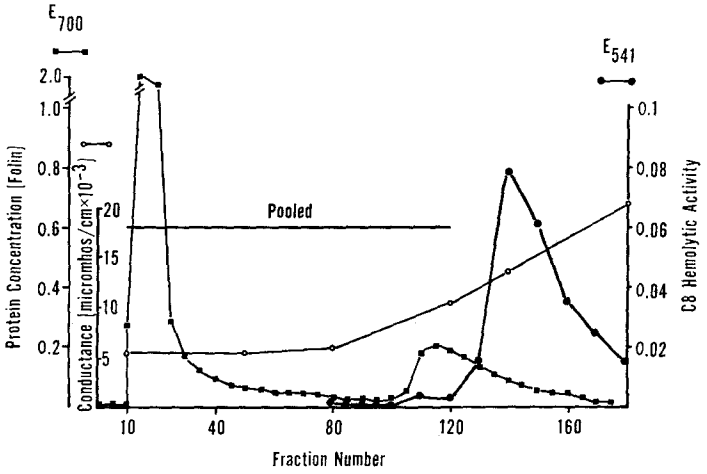

FIG. 1. Preparation of the reagent utilized for the conversion of EACl–2 to EACl–7. Elution profile obtained after chromatography of 60 ml of human serum on CM-cellulose at pH 6, ionic strength 0.1, in phosphate buffer. NaCl concentration gradient was started at fraction No. 50. Fractions were pooled as indicated, to include C3, C5, C6, and C7, but not C8.

hr at 4° C (starting buffer) and was applied to a 4.5 × 60 cm column of carboxymethyl (CM)-cellulose which was equilibrated with starting buffer. After the column was washed with 1000 ml of this buffer, an NaCl concentration gradient was begun, the concentration of NaCl in the limiting buffer being 1.8%. The material which emerged before the C8 activity was pooled (Fig. 1) and immediately frozen without concentration.

EAC1, 4, 2 was incubated at a concentration of 5×10^8 cells per ml with an equal volume of C3–7 reagent, restored to isotonicity with dextrose, and supplemented with 5–10 μg of purified C5 per ml of reaction mixture. After 45 min of incubation at 37° C the cells were washed at least three times, resuspended in GVB, and adjusted to a concentration of 5×10^8 cells per ml. These cells could not be lysed by C9 alone, but they lysed completely within a few min on addition of C8 and C9.

C8 activity was assayed with 10^8 EAC1–7 cells and an excess of purified C9 in a total reaction volume of 0.6–0.8 ml. The reaction was performed at 37° C and stopped usually after 30 min by addition of 2 ml of cold saline and immediate centrifugation.

Procedure of Isolation of C8

Fractionation of Serum with Rivanol.—Usually, 2000 ml of pooled serum served as starting material for one C8 preparation. The pH of the pool was adjusted to 7.5 with 1N NaOH, and

[2] Nomenclature used conforms with the recommendations of the W. H. O. Committee on Complement Nomenclature (6).

4 × 500 ml were filled into 1000 ml plastic containers to which an equal volume of 1% rivanol in water was added with constant stirring at 0° C. The heavy yellow-green precipitate was sedimented by centrifugation at 500 *g* for 30 min in the cold and washed with 1000 ml of 0.5% rivanol solution in water. Approximately 100 ml of a 3% NaCl solution in water was then added to each container and a period of 2 hr at 4° C was allowed for dissolution of the protein precipitate. Under these conditions, most of the rivanol is insoluble and may be removed by centrifugation for 30 min at 500 *g*. The protein solution was dialyzed against 2 × 20 liters of phosphate buffer, pH 6.0, ionic strength 0.1, for 24 hr (starting buffer).

CM-Cellulose Chromatography.—Approximately 850 ml of packed CM-cellulose in a 4.5 × 60 cm column was equilibrated overnight with starting buffer (see above). 400 ml of the dialyzed rivanol fraction was applied to the column, followed by a wash with 1000–1500 ml of starting buffer. The column was eluted with 3 liters of a linear NaCl concentration gradient in which the limiting buffer consisted of 1.5 liter starting buffer adjusted to a conductance of 35 millimhos by addition of 27 g NaCl. The flow rate was adjusted to 60 ml per hr and 15 ml fractions were collected. The C8 activity containing fractions were pooled and concentrated to 6–7 ml by ultrafiltration using a UM10 membrane (Amicon Corp., Cambridge, Mass.).

Pevikon Block Electrophoresis.—6–7 ml of the concentrated material was applied at the center of a 1 × 18 × 50 cm block of Pevikon in phosphate buffer, pH 6.0, ionic strength 0.05. After electrophoresis at 3.5 v/cm for 40 hr, the block was cut into 1.25 cm segments and eluted with electrophoresis buffer. The fractions containing C8 activity were pooled and concentrated to 2 ml as described above.

Gel Filtration.—For preparative purposes as well as for estimation of the diffusion coefficient of C8, a 3 × 100 cm column of Sephadex G-200 was employed which was equilibrated with the limiting buffer used for CM chromatography (see above). The sample volume was 2 ml, the flow rate was adjusted to 8–10 ml per hr and 2 ml fractions were collected. The C8 containing fractions were pooled and concentrated by ultrafiltration to 2–3 ml.

Absorption with Insolubilized Anti-γG-Globulin.—5 ml of a rabbit antiserum to human γG-globulin was mixed with 5 ml 0.2 M acetate buffer, pH 5.2, and the protein was insolubilized by addition of 0.8 ml ethyl chloroformate (9). The mixture was agitated with a magnetic stirrer for 15 min during which time the pH was maintained between 4.5 and 5.0 by addition of 1 N NaOH. After 30 min at room temperature, the solid protein was washed 20 times with 50 ml portions of phosphate-buffered saline, pH 7.2. It was then washed with 5 × 50 ml 0.1% sodium carbonate, 10 × 50 ml 0.2 M glycine buffer, pH 2.2, and sufficient phosphate buffered saline to reach pH 7.2. It was finally suspended in 10 ml saline and centrifuged at 20,000 rpm for 1 hr in a No. 40 rotor and a Spinco L2 machine. The fluid was discarded, the walls of the tube dried with filter paper and the wet protein (400 mg) was suspended in 2.5 ml of C8 preparation (200–600 μg/ml) after the gel filtration step. The suspension was kept overnight at 4° C and then subjected to ultracentrifugation as described above. 2.2 ml of supernatant was recovered with a Pasteur pipette. The extensive washing of the immune-absorbent was necessary because ethyl chloroformate in a dilution of 1:40,000 was found to inactivate C8 completely.

The final product was stored in phosphate buffer, pH 6.0, ionic strength 0.1, containing 0.3 M NaCl at −70° C after quick freezing in liquid nitrogen.

To prevent bacterial growth during the isolation procedure, which usually lasts 12–15 days, all buffers used for chromatography and gel filtration contained 5×10^{-2} M chloramphenicol and 2.5×10^{-5} M kanamycine sulfate (Bristol Laboratories, Syracuse, N.Y.).

Sucrose Density Gradient Ultracentrifugation.—Linear sucrose density gradients (7–31%) in phosphate buffer, pH 6.0, ionic strength 0.1, were prepared with a Buchler automatic density gradient device. Ultracentrifugation was performed for 12 hr at 40,000 rpm and 4° C in a Spinco L2 centrifuge employing an SW-50 rotor. Fractions were collected with the Buchler gradient fractionation device.

Analytical Polyacrylamide Gel Electrophoresis.—The method described by Davis (10) was used. A 5% acrylamide concentration was found to be most suitable to study the migration of

C8. Polymerization of the sample-containing gel and electrophoresis were carried out at 4° C. A current of 2.5 ma per gel was applied and electrophoresis was terminated when the bromphenol blue marker had traveled 5 cm from the cathodal end of the separating gel. For activity-protein correlation experiments, equal portions of C8 were applied simultaneously to two parallel polyacrylamide gels, one gel was subsequently stained, while the other was cut into 2.5 mm segments which were minced in 0.2 ml of GVB. After standing for at least 2 hr at room temperature, the eluates were analyzed for hemolytic activty.

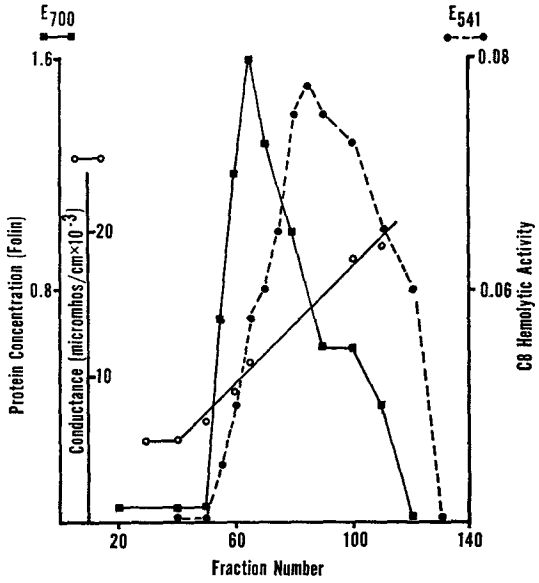

FIG. 2. Second preparative step of the C8 isolation procedure: chromatography on CM-cellulose of rivanol-precipitable fraction of human serum at pH 6, inoic strength 0.1, in phosphate buffer. 100 g of protein in 400 ml were applied to the column. The portion of the chromatogram representing protein which was not adsorbed (99% of the applied material) is not shown. Elution of C8 activity and the adsorbed serum protein was accomplished by a NaCl concentration gradient. Fractions 60–135 were pooled and concentrated for electrophoresis.

Immunochemical Analyses.—Immunologic analysis of the C8 preparations was performed in Ouchterlony plates using rabbit antisera to C2 (11), C3 (7), C4 (12), C5 (4) and the followig antisera purchased from Behringwerke AG, Marburg-Lahn, West Germany: anti-γG, -γA, -γM, -β-lipoprotein, -transferrin, -fibrinogen, -haptoglobin, -α2M and anti whole human serum. An antiserum to human C8 was prepared as described subsequently. Immunoelectrophoresis was performed according to Scheidegger (13) using either agar or agarose. In experiments where C8 hemolytic activity was correlated with the precipitin arc developed with an anti-C8 serum half of the microimmunoelectrophoresis gel was cut into 2.5 mm segments which were eluted with GVB for activity determination.

C8 Protein Determination.—This was carried out with the Folin method using, arbitrarily, human γG-globulin as standard.

RESULTS

Isolation of C8.—Since earlier experiments had shown that only 20% of the C8 activity precipitated together with the euglobulins while 80% remained in the pseudoglobulin fraction (14), whole serum was used as starting material (140 g protein). Upon addition of rivanol, C8 activity was precipitated together

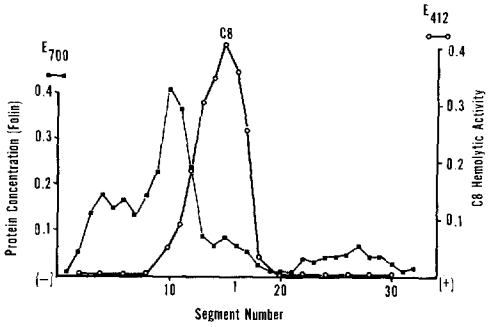

FIG. 3. Third step of C8 isolation: preparative block electrophoresis in phosphate buffer, pH 6.0, ionic strength 0.05, using Pevikon C-870 as supporting medium. 8 ml containing 800 mg of protein and C8 activity obtained by CM chromatography were applied as indicated by the arrow and subjected to electrophoresis for 40 hr at a potential gradient of 3.5 v/cm. Eluates of segments 11–18 were pooled and concentrated for Sephadex filtration.

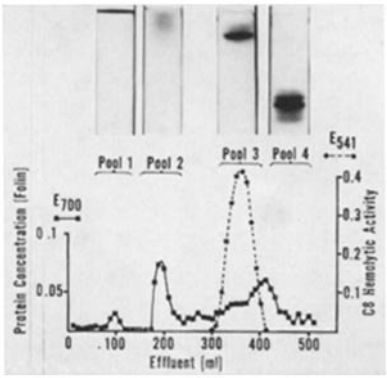

FIG. 4. Fourth step of C8 isolation: filtration on Sephadex G-200 in phosphate buffer, pH 6.0, ionic strength 0.1, containing 0.3 M NaCl. 2 ml containing 50 mg of protein and C8 activity recovered from the Pevikon block were applied. Fractions containing C8 activity were combined (pool 3) and concentrated for removal of residual γ-globulin by immune-adsorption. The concentrated material gave one major line on polyacrylamide gel electrophoresis as shown in the upper portion of the figure. For comparison, disc electrophoresis patterns of adjacent pools are also shown.

with about 70% of the serum proteins, most of the γ-globulin and transferrin staying in solution. This step was desirable because γG-globulin proved difficult to separate from C8 and it also resulted in a convenient decrease in volume. After removal of the rivanol and dialysis against starting buffer for chromatography, approximately 100 g of protein in 400 ml were applied to a CM-cellulose column. Washing of the column with starting buffer eliminated about

TABLE I

Final Purification of C8 by Absorption with Insolubilized Rabbit Anti-Human γG-Globulin

Preparation No.	Amount of protein (μg)	
	Before	After
38	4600	1370
40	5400	1240
43	1220	700
44	575	300

TABLE II

Yield and Degree of Purification of C8 Isolated from Human Serum

	Amount of protein		No. of effective C8 molecules	
	Total	Required for 63% lysis*	Total	Per μg
	mg	*ng*		
Serum	140,000	500	14×10^{15}	1×10^{8}
Isolated C8 (No. 38)	1.4	0.1	7×10^{14}	5×10^{11}
Yield	0.001%		5%	
Purification		5000 ×		5000 ×

* 5×10^{7} EAC1-7 cells.

99% of the protein without interfering with absorption of most of the C8 activity. The latter was eluted by NaCl gradient as shown in Fig. 2, and approximately 800 mg of protein containing C8 was recovered. This material was concentrated to 8 ml and subjected to Pevikon block electrophoresis at pH 6.0 which resulted in removal of more than 90% of non-C8 protein, the latter migrating cathodally and anodally from the point of application. As shown in Fig. 3, C8 activity was recovered from segments adjacent to the origin. The pooled fractions contained approximately 50 mg of protein which was passed through a Sephadex G-200 column. The elution profile and polyacrylamide gel electrophoresis patterns corresponding to the major peaks are shown in Fig. 4. C8 activity was found to distribute between the theoretical positions of albumin and γG-globulin and to be associated with approximately 5 mg of protein, or 10% of the material applied to the column. Although C8

was retarded on Sephadex G-200 relative to the peak of γG, the C8 pool was found to be heavily contaminated with γ-globulin when examined immunochemically. Instead of attempting further separation by recycling on Sephadex, immunoabsorption was introduced as final purification step in view of the relatively small amount of residual protein available. Rabbit antiserum to human γG-globulin was insolubilized by treatment with ethyl chloroformate according to Avrameas and Ternynck (9) and the washed, polymerized protein was used as immune-adsorbent. Upon absorption of the C8 preparation after the Sephadex G-200 step, one-half to one-fourth of the protein was recovered (Table I) and about 75% of the C8 activity. By Ouchterlony test with anti-human γG-globulin there was no residual γG detectable in the absorbed C8 (Fig. 6).

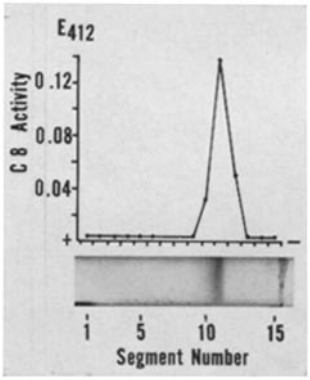

FIG. 5. Analysis of final product of C8 isolation procedure by disc electrophoresis and correlation between protein and C8 activity distribution. 25 μg of isolated C8 was applied to each of two gels. After electrophoresis, one gel was stained for protein (below) and the other was cut into 2.5 mm segments for elution and localization of C8 activity (above).

The yield of C8 activity varied between 0.25 and 5% of the activity present in serum. Table II lists yield of activity and of protein for preparation 38. Purification in this case was calculated to be 5000-fold compared to serum. Using the data of Table II and assuming that all of the final product represented active C8 protein, the total amount of C8 in 2 liters of serum was estimated to be 28 mg or 0.0002% of the serum protein and the concentration of C8 in serum to be 14 μg/ml.

Electrophoretic and Immunochemical Examination of Isolated C8.—25 μg of isolated C8 was applied to each of two polyacrylamide gels and the two samples were subjected simultaneously to disc electrophoresis under identical conditions. One gel was stained for protein, the other was analyzed for the distribution of C8 hemolytic activity. As illustrated in Fig. 5, the position of the activity coincided with that of the protein. Additional protein bands could not be detected.

Fig. 6 depicts the results of an analysis of C8 by the double diffusion-in-gel technique. Gamma globulin could not be detected with a potent rabbit anti-γG in preparations of C8 which had been subjected to the final purification step. Similarly, negative reactions were obtained with the following antisera

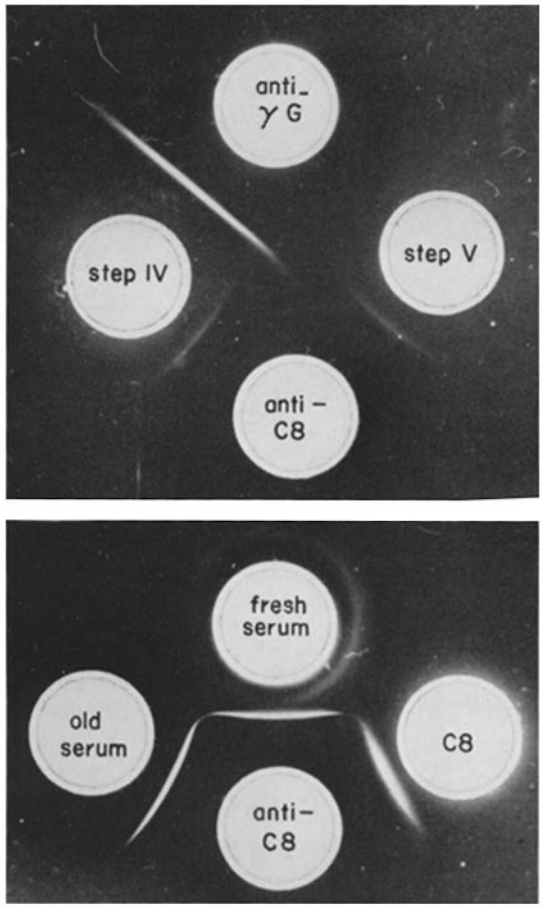

FIG. 6. Analysis of C8 by Ouchterlony test. Above: C8 before (step IV) and after (step V) absorption with polymerized anti-γG. Below: Comparison of isolated C8 (50 μg) with C8 in fresh serum and in serum stored for 1 month at 4°C.

(not shown): anti-γA, anti-γM, anti-fibrinogen, anti-transferrin, anti-β-lipoprotein, anti-α_2-macroglobulin, anti-haptoglobin, anti-C2, anti-C3, anti-C4, and anti-C5. In contrast, a precipitin line was observed with an anti-C8 which was slightly concave toward the antigen well. The anti-C8 also produced

a line with whole human serum which fused with the C8 line, indicating that anti-C8 can detect C8 in unfractionated serum.

The immunoelectrophoretic appearance of C8 is depicted in Fig. 7 which shows that the mobility is dependent on the type of supporting gel used.

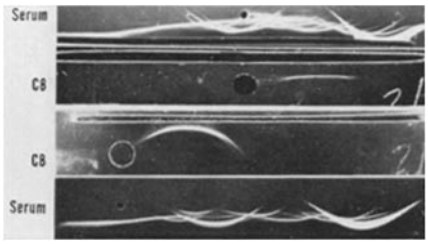

FIG. 7. Immunoelectrophoretic representation of isolated C8. The upper two patterns were obtained using agar and the lower two using agarose as supporting medium. For reference purposes the pattern of whole human serum was included. The latter was developed with a rabbit anti-whole human serum, and the C8 precipitin line with a rabbit anti-human C8. The anode was at the right. Note that in agar C8 migrates as an α-globulin and in agarose as a γ_1-β_2 globulin. A precipitin arc corresponding to C8 could not be seen in the pattern of whole serum.

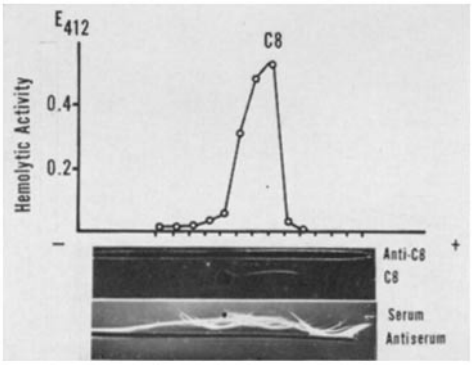

FIG. 8. Comparative distribution of C8 activity and of immunochemically detectable C8 protein after agar gel electrophoresis. Half of the agar plate was cut into 2.5 mm segments for activity assay (above) and the other half was developed with an antiserum to human C8 (center). For comparison, the immunoelectrophoretic pattern of whole human serum is also shown (below).

Whereas in agarose it migrates, as in Pevikon, like a fast γ-globulin, C8 behaves in agar like an α_2-globulin. A similar anomalous behavior has previously been reported for isolated C2 (11). C8 hemolytic activity was eluted exactly from the area of the gel which was occupied by the C8 precipitin arc, i.e., from the fast γ-globulin position if agarose was used and from the α_2 region if the

experiment was performed in agar. The comparative distribution of the C8 precipitin arc and of C8 hemolytic activity in agar is shown in Fig. 8.

Physicochemical Characterization of C8.—The sedimentation coefficient of purified C8 was estimated by sucrose density gradient ultracentrifugation using

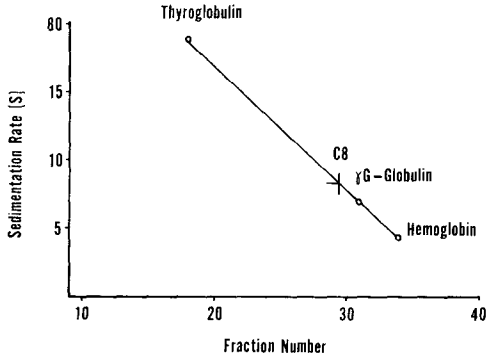

FIG. 9. Determination of the sedimentation coefficient of C8 after ultracentrifugation in a linear sucrose density gradient in the presence of three reference substances (thyroglobulin, 19S; γG-globulin, 7S; hemoglobin, 4.5S).

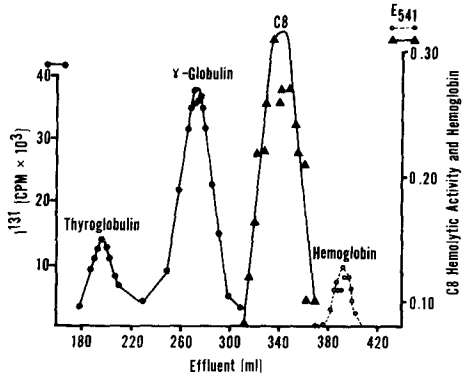

FIG. 10. Elution diagram of C8 obtained after filtration on a Sephadex G-200 column (3 × 100 cm) in the presence of three reference substances. Phosphate buffer, pH 6.0, ionic strength 0.1, containing 0.3 M NaCl was used.

reference substances with a known *s* rate. The graphical evaluation of one of these analyses is shown in Fig. 9. The diffusion coefficient was determined using the same reference substances by filtration through a Sephadex G-200 column (15). The elution profile of C8 and reference proteins is illustrated in Fig. 10 and the graphical analysis of this experiment in Fig. 11. It will be noted that although the *s* rate of C8 is greater than that of γG-globulin, C8 is eluted

on gel filtration later than γG. The average values of the molecular parameters obtained from replicate experiments and the calculated molecular weight are listed in Table III.

Hemolytic Efficiency of Isolated C8.—When various preparations of isolated C8 were analyzed with EAC1–7 cells for hemolytic activity, linear dose re-

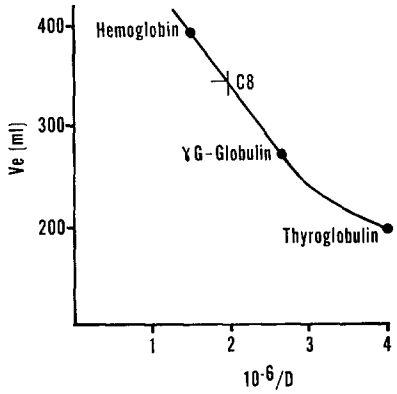

FIG. 11. Estimation of the diffusion coefficient of C8 after gel filtration on Sephadex G-200 in the presence of three reference substances (thyroglobulin, D = 2.5 × 10^{-7} cm^2/sec; γG-globulin, D = 3.8 × 10^{-7} cm^2/sec; hemoglobin, D = 6.8 × 10^{-7} cm^2/sec). The elution volumes (Ve) are plotted against the reciprocal of D according to Andrews (15).

TABLE III

Physicochemical Properties of Isolated C8

Sedimentation coefficient	8.5S
Diffusion coefficient	5.0 × 10^{-7} cm^2/sec
Molecular weight*	153,000
Frictional ratio‡	1.2
Electrophoretic mobility§	γ1

* Assuming $\bar{v}$ = 0.73.

‡ Determined nomographically (23).

§ In Pevikon at pH 6.0 and 8.6.

sponse curves were invariably obtained when the data were plotted in terms of the reciprocal of the negative natural logarithm of the proportion of unlysed cells versus the reciprocal of the C8 dilution. An example of a dose response curve is given in Fig. 12, which clearly demonstrates the existence of proportionality between input of C8 and the final degree of hemolysis. The preparation used for this experiment had a protein concentration of 60 μg/ml. A $1/-ln(1-y)$ value of unity, which corresponds to 63% lysis, was obtained with a 44,000-fold dilution of this C8 preparation. The actual amount of protein

added to 5×10^7 EAC1–7 cells at this dilution was calculated to be 227 picograms or 1.06×10^9 molecules. Thus, according to the one-hit theory of immune hemolysis (16), 21 molecules of C8 per cell were required to produce one "hemolytically effective molecule".

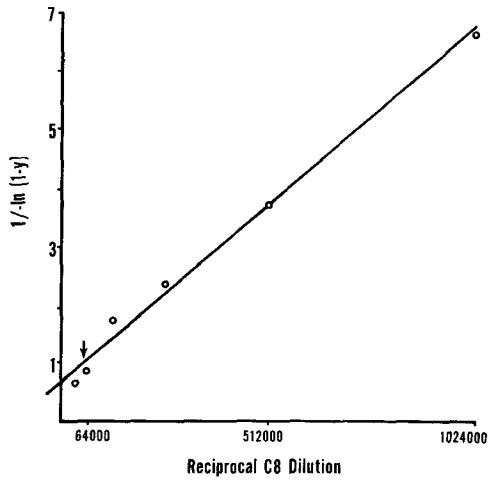

FIG. 12. Dose response for highly purified C8: reciprocal of average number of C8 sites per cell $[-ln(1-y)]$ versus reciprocal of C8 dilution. A$[-ln(1-y)]$ value of unity was obtained at a 44,000-fold dilution which corresponds to 227 picograms of C8 protein per 5×10^7 cells.

TABLE IV

Hemolytic Efficiency of C8 Expressed in Molecules Per Cell Required for 63% Hemolysis

Preparation No.	Input	Specific uptake
38	8	—*
37, 38, 39	21	—
40a	16	—
40b	80	—
40b(^{125}I)	160	34
40b1(^{125}I)	560	24
43	15	—

* —, not done.

Comparing various preparations with respect to their hemolytic efficiency, considerable variation was encountered. As listed in Table IV, the number of C8 molecules required to produce one hemolytically detectable C8 site ranged from 8–560, and the hemolytic efficiency from 12–0.018%. The possibility was considered that the observed differences were due to the presence in the C8 preparations of varying amounts of hemolytically inactive material. While

this possibility could not be ruled out, it is emphasized that the immunoelectrophoretic appearance of several preparations tested was as illustrated in Fig. 7 and did not indicate heterogeneity. That the active and inactive forms of complement proteins may have a different immunoelectrophoretic behavior has previously been demonstrated for C2 (11), C3 (7), C4 (12), and C5 (17). An alternative explanation offered itself: since the data of Table IV were ob-

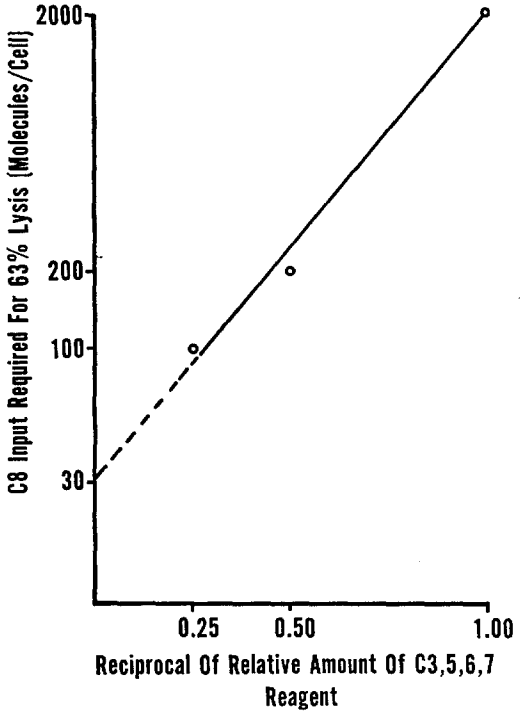

FIG. 13. Dependence of the apparent hemolytic efficiency of C8 upon the reactivity of the assay cells (EAC1–7). The same C8 preparation was assayed with three different batches of EAC1–7 cells which differed in the number of sites on their surface capable of reacting with C8.

tained at different days with different preparations of assay cells, the encountered variation in the hemolytic efficiency of C8 preparations might be a function of the reactivity of the assay cells. Accordingly, several batches of EAC1–7 cells were prepared from EAC1,4,^{oxy}2 using different amounts of the same C3,5,6,7-reagent. These cells, which were qualitatively identical but differed in number of C3,5,6,7 sites per cell, were reacted with the same preparation of C8 and the number of C8 molecules per cell required to produce 63% lysis (in the presence of C9) was determined for each batch of assay cells. Fig. 13 shows the marked dependence of the apparent hemolytic efficiency of C8 upon

the reactivity of the assay cells. It is therefore concluded that the variation in hemolytic efficiency encountered among different C8 preparations is, in part, due to variations in the functional condition of the assay cells.

Also shown in Table IV is that only a fraction of the radiolabeled molecules required to achieve 63% lysis became specifically bound to the cell surface. In two experiments with ^{125}I–C8, the uptake was respectively 4.5 and 21% of input. The average of the four best input values (8, 21, 16, and 15 molecules per cell) is 15 C8 molecules per cell at 63% lysis. The average uptake in the two experiments with radioactive C8 is 12.5%. Assuming that these values are representative for C8 under optimal experimental conditions, one hemolytically effective C8 site may be produced by 1.9 molecules of C8 protein.

DISCUSSION

To date, isolation and identification of the following complement proteins of human serum have been reported: C1q (18), C1s (19), C2 (11), C3 (4), C4 (12), C5 (4), and C9 (3). To this group of purified proteins is now being added C8, the eighth component of human complement. It is an immunochemically definable γ_1-globulin which has a calculated molecular weight of 153,000 and which is unrelated to either γG, γA, or γM. The protein may be obtained in homogeneous form as judged by polyacrylamide and immunoelectrophoresis. Its concentration in serum is relatively low; by hemolytic activity measurements it was estimated to be 10–20 μg/ml; by a semiquantitative immunodiffusion test it appeared to be at least twice this value. Accordingly, the yield of purified material was low and was comparable with that of other trace proteins of serum such as C2 (11). But unlike some other complement proteins, notably C2, C3, C4, and C5, it has been found to exhibit a remarkable stability and resistance to "spontaneous" inactivation during the isolation procedure.

The presented method of isolation was elaborated during the past 3 yr and in its final form has been found in this laboratory to be highly reproducible. The use of an immune-adsorbent in the final purification step represents a departure from the exclusive use of physicochemical methods for the purification of other complement proteins. It was dictated by the encountered difficulty to separate C8 from a physicochemically similar species of γG-globulin and by the small amounts of protein available for final purification. It is possible that recycling on Sephadex G-200 or chromatography on phosphoric acid cation exchange cellulose might produce similar results.

Although the yield of C8 activity in the isolated material was at best 5% relative to whole serum, the degree of purification was 5000-fold. The hemolytic efficiency varied among different preparations obtained, however, in most cases it was exceedingly high compared with other complement components except C1. Whereas one C1 molecule was reported to be sufficient to produce one hemolytically detectable site (20), the number of C2, C3, C4, and C5

molecules required for the formation of a hemolytic site is under optimal conditions, respectively, 2600, 3000, 300, and 280 (21). Since only a fraction of the molecules entering the complement reaction achieves binding to the surface of the target cell, the hemolytic efficiency of specifically cell-bound complement molecules is considerably greater than the numbers cited above. For C5, for example, 3–5 specifically cell-bound molecules suffice to produce one hemolytically effective site (22). The evidence accumulated in the present study indicates that 1–2 specifically bound C8 molecules may be equivalent to one "effective C8 molecule" in the sense of Mayer's one-hit theory (16). The underlying calculations are predicated on the assumption that all or the major portion of a C8 molecule becomes cell-bound during the C8 reaction step. Thus, the complement component, which is presently considered directly responsible for the production of cell membrane damage, approaches in its specifically cell-bound form the one molecule–one lesion relationship.

SUMMARY

A method was described allowing the isolation of the eighth component of complement, C8, from human serum. C8 is an immunochemically definable, heretofore unrecognized serum protein with a molecular weight of approximately 150,000 and an electrophoretic mobility of a γ_1-globulin. It may be obtained with 5% yield as a 5000-fold purified, physicochemically and immunochemically homogeneous protein. Under optimal experimental conditions, a few molecules of C8 were found sufficient for lysis of a cell.

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