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ISOLATION OF THE TERMINAL COMPLEMENT COMPLEX FROM TARGET SHEEP ERYTHROCYTE MEMBRANES

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SUMMARY

(1) Membranes from sheep erythrocytes lysed with antibody and human complement were solubilized in Triton X-100 and subjected to isoelectric focusing in polyacrylamide gels containing 1 % Triton X-100. Membrane-bound serum proteins were located in the gels by subsequent immunoelectrophoresis against antisera to human serum proteins. Monospecific antisera against C9 and C5 were used to locate the terminal complement complex, which is not dissociated by Triton X-100. The complex focused between pH 5.8 and pH 6.5 and was separated from the bulk of other membrane-bound serum proteins, which focused at pH ranges below than 6.0.

(2) In a second step, proteins electrophoretically eluted from the gel sections containing the terminal complement complex were chromatographed on Sepharose 6B equilibrated with 0.05 % Triton X-100. Fused rocket immunoelectrophoresis was used to monitor separations. This step separated the terminal complement complex from the remaining contaminating proteins. The complex eluted in a broad peak corresponding to a molecular weight range of 800 000–4 000 000.

(3) The terminal complement complex thus obtained migrated with α-mobility and yielded a single precipitation arc in crossed immunoelectrophoresis using polyvalent antisera to human serum proteins. A distinct precipitate was obtained with monospecific anti-C9. The presence of C5 and C6, in complex with one another and with C9 was demonstrable by immuno-double-diffusion. No immunoprecipitate was obtained with antisera to sheep erythrocyte membrane proteins.

(4) Dodecyl sulfate gel electrophoresis of the complex revealed seven protein bands of 190 000, 160 000, 115 000, 93 000, 85 000, 68 000 and 60 000 daltons. Planimetric quantitation of densitometric scans gave a molar ratio of approx. 0.7 : 0.3 : 1 : 1 : 1 : 2 : 1 for these bands, respectively. All bands stained faintly with periodate-Schiff. Two-dimensional dodecyl sulfate gel electrophoresis showed that the first two bands (190 000 and 160 000 daltons, probably C5b and C5c) represented proteins possessing more than one peptide chain linked by disulfide bonds. The main subunit for both bands was a protein of approximatley 68 000 daltons. Band 5 (83 000 daltons, probably C8α) was split into two peptide chains of approximately 68 000 and 15 000 daltons. The other components were not affected by dithiothreitol treatment.

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(5) The dodecyl sulfate gel electrophoretograms obtained were very similar to that described by Kolb and Müller-Eberhard (Kolb, W. P. and Müller-Eberhard, H. J. (1975) J. Exp. Med. 141, 724–735) for the terminal complement complex isolated from inulin-activated serum. However, certain minor but consistent deviations were observed. A preliminary correlation of the electrophoretograms is presented.

INTRODUCTION

Recent studies on the final stages of complement lysis have underlined the importance of the five terminal complement components (C5–C9), whose assembly on target membranes leads to cell lysis [2–6]. Previous communications have described the mode of interaction of these five components in solution [2–6], and stable complex formation between C5, C6 and C9 on target membranes has also been documented immunochemically [7].

It has been hypothesized that the terminal complement complex itself may be directly responsible for membrane pertubation, either by insertion of an exposed hydrophobic, lytic "tail" [8] or by forming hydrophilic channels through membranes [9]. We have recently presented data that indicate that the complement complex may indeed be anchored to membrane core components; this could be directly responsible for the complement-induced changes in membranes observable by freeze-etch electron microscopy [10], and might also lead to disturbances in membrane structure which cause cell lysis.

A prerequisite to further studies on the mode of interaction between terminal complement components and target membranes is the isolation and further characterisation of the macromolecular complex. Kolb and Müller-Eberhard [1] have described a method of purifying the complex from inulin-activated human serum. They were also able to identify the various complement components present in the complex by dodecyl sulfate gel electrophoresis. However, the complex obtained from inulin-activated serum does not possess lytic properties and need not be identical to the complex assembled on target membranes during cell lysis. In this communication, a simple, rapid procedure is described for isolating the terminal complement complex from target membranes. The available data indicate that it is very similar, but not identical, to the complement complex obtained from inulin-activated serum.

MATERIALS AND METHODS

Unless otherwise stated, all chemicals and biochemicals were obtained from Serva (Heidelberg), Boehringer (Mannheim) and Merck (Darmstadt). Sheep erythrocytes stored in acid-citrate dextrose for 4–21 days were used in all experiments. Fresh human serum was our source of complement. Rabbit anti-sheep erythrocyte antiserum ("Ambozeptor") and monospecific antisera against human complement components C4 (batch 2538 L), C5 (batch 2540) and C9 (batch 2602 K) were purchased from Behring (Marburg). Monospecific rabbit anti-human C3 (β1c andβ1a) immunoglobulins were a gift of Dakopatts A/S, Copenhagen. Monospecific anti-rabbit C6 antisera, which cross-react with human C6 [11] were a gift of U. and K. Rother, Heidelberg.

Rabbit antisera against human serum proteins were obtained from Behring (Marburg) and Dakopatts A/S (Copenhagen). Preliminary experiments showed that the sharpness of the immunoprecipitates obtained for the terminal complement complex varied greatly depending on the antiserum batch used. In current experiments, immunoglobulins coded 100 SA and 100 SF from Dakopatts A/S are routinely utilized.

Agarose was purchased from Litex (Glostrup, Denmark, batch 131 A). Triton X-100 (scintillation grade) was purchased from Serva (Heidelberg). Ampholines of pH range 3.5–10.0 were obtained from LKB, and Sepharose 6B from Pharmacia (Uppsala, Sweden).

Complement lysis. Sheep erythrocytes (20 ml packed volume) were washed three times in isotonic saline and suspended in 200 ml of veronal-buffered saline (0.15 mM Ca^{2+} and 1.0 mM Mg^{2+}). Anti-sheep erythrocyte antiserum (2 ml) was added and the suspension kept at 37 °C for 20 min with gentle mixing. Human serum (100 ml) was then added and complement lysis was complete within 3 min at 37 °C. Membranes were subsequently centrifuged and washed 4 times with ice-cold 5 mM phosphate (pH 8.0) in a Sorvall centrifuge (8 min, 25 000 × *g*, rotor SS 34). The membrane suspensions obtained after the final wash contained 9–10 mg protein/ml.

Membrane solubilisation. Solubilisation with Triton X-100 was performed as in ref. 12. The samples obtained will be referred to as the "Triton extracts".

Isoelectric focusing. Triton extracts were made 15–20 % in sucrose and directly electrofocused on polyacrylamide gel rods (0.5 cm diameter, 9 cm height) containing 2 % ampholines, 1 % Triton X-100 and 10 % sucrose. Gels were prepared as in ref. 13, except that water was substituted for urea and 20 % Triton X-100 was added to a final concentration of 1 % (v/v). A detailed description of the procedure for preparing gels has been given in ref. 14.

For electrofocusing, 350–400 μl of Triton extract (approximately 2 mg protein) were applied to each gel. Focusing conditions and pH gradient measurements were as in ref. 12.

Isoelectric focusing linked to immunoelectrophoresis. After electrofocusing, gels were removed from the glass tubes, sliced longitudinally and both halves placed side by side on 0.5 mm thick agarose slabs (10 × 8 cm). A second layer of agarose (1.5 mm thick) was then cast to mould in the polyacrylamide gels. After gelation, the agarose on one side of the polyacrylamide gel was removed and replaced with a 1.5 mm agarose layer containing antibodies, as used in normal rocket immunoelectrophoresis [15]. Electrophoresis into the antibody-containing agarose was carried out in an LKB Multiphor apparatus (model 2117) at 1.5 $V \cdot cm^{-1}$ for 24 h (15 °C). The agarose contained 1 % Triton X-100 in all experiments.

Elution of proteins from electrofocused gels. Gel slices were placed on a 5 cm sintered glass funnel whose spout had been sealed by attaching a closed dialysis bag and then filled with 50 mM glycine/19 mM Tris (pH 8.6). The bag was immersed in 400 ml of the same buffer. The upper (open) end of the funnel containing the gel slices was filled with buffer and made the cathode. Electrophoresis was performed at 500 V for 12 h in a cold room (5 °C). The dialysis bag was subsequently punctured, the fluid in the funnel collected, made 0.1 % with Triton X-100 and then concentrated over Amicon PM 10 Diaflo membranes.

Sepharose 6B chromatography. The concentrated samples eluted from electro-

focused gels were subjected to descending chromatography on Sepharose 6B. The column (1.5 cm diameter, 90 cm height) was equilibrated and eluted at room temperature with 50 mM NaCl/10 mM Tris (pH 8.2) containing 15 mM NaN_3 and 0.05 % Triton X-100. Samples applied were 2–3 ml, from which 4-ml fractions were collected at a flow rate of 5.32 ml/h. Blue Dextran 2000, IgM, IgG, hemoglobin and *p*-nitrophenol were used as calibration standards.

Dodecyl sulfate gel electrophoresis. Samples were made 4 % in sodium dodecyl sulfate, heated at 100 °C for 5 min, and electrophoresed on polyacrylamide gels containing 1 % sodium dodecyl sulfate in 40 mM Tris/20 mM sodium acetate/2 mM EDTA (pH 7.4) as described in ref. 16. The electrophoresis buffer contained 1 % dodecyl sulfate. Gels were 5 % acrylamide cross-linked to 2.5 % with *N,N'*-methylenebisacrylamide (0.5 cm diameter, 9 cm height). Electrophoresis was performed at room temperature at 5 mA/gel and the tracking dye, Pyronin G, allowed to migrate 7.0 cm into the gel. Staining with Coomassie brilliant blue and periodate-Schiff was as in ref. 16. Relative mobilities and proportions of protein bands were determined by densitometric scanning at 620 nm after staining with Coomassie brilliant blue using a Gilford spectrophotometer (Model 240) equipped with a synchronous linear transport (Model 2410-5) and an electronic integrator (Hewlett-Packard, Model 3370-B7). Gels were calibrated with the following standard proteins of known molecular weight [17, 18] : rabbit immunoglobulin (155 000), *E. coli* β-galactosidase (130 000), rabbit muscle phosphorylase A (93 000), bovine liver catalase (59 000) rabbit immunoglobulin G light chains (23 500) and horse skeletal muscle myoglobin (17 200).

Two-dimensional dodecyl sulfate gel electrophoresis. The procedure was basically as described in ref. 19, using a multislab apparatus [20] with minor modifications [21]. Disulfide cleavage between first- and second-dimension electrophoresis was effected by dialysing gels against 120 mM dithiothreitol for 60 min in electrophoresis buffer containing 1 % dodecyl sulfate at room temperature.

Immunoelectrophoretic techniques. Crossed immunoelectrophoresis was performed as in ref. 22. Fractionations over Sepharose 6B were monitored by fused rocket immunoelectrophoresis [23]. The buffer used in all experiments was 100 mM glycine/38 mM Tris (pH 8.7). Agarose was also dissolved in this buffer and made 1 % in Triton X-100 [24, 25].

Experimental protocol. Approximately 15 ml of Triton extract (5 mg protein/ml) were electrofocused on 40 polyacrylamide gels. After isoelectric focusing, the gel sections containing the complement complex (determined by immunoelectrophoresis against anti-C9/anti-C5) were cut out and eluted electrophoretically. The sample was then made 0.1 % in Triton X-100, concentrated to approximately 3 ml, and chromatographed over Sepharose 6B. The fractions containing the terminal complement complex (as monitored by fused rocket immunoelectrophoresis) were pooled and concentrated to 1.5–2.0 ml.

RESULTS

Isoelectric focusing of membrane-bound serum proteins

As previously demonstrated with immunochemical methods, a large number of serum proteins are present on complement-lysed sheep erythrocyte membranes [26]. The majority represent non-specifically absorbed entities, but certain complement components can be readily identified in crossed immunoelectrophoresis [26].

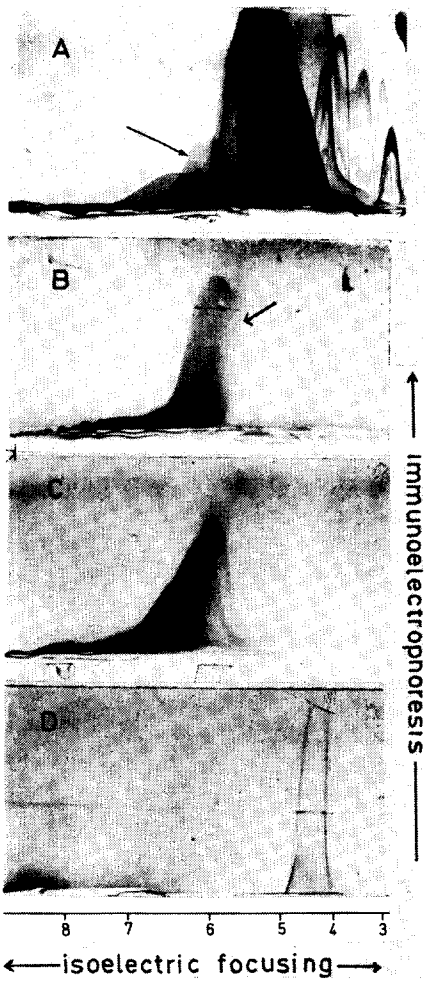

Fig. 1. Isoelectric focusing of complement-lysed membranes (A-C) and native serum (D) in polyacrylamide gels containing 1 % Triton X-100 (left to right), followed by immunoelectrophoresis into antibody-containing agarose (bottom to top). Agarose gels contain (A) 30 μl/cm^2 immunoglobulins to human serum proteins, (B) 10 μl/cm^2 monospecific anti-C9, (C) 10 μl/cm^2 anti-C9 plus 10 μl/cm^2 anti-C5, (D) 5 μl/cm^2 anti-C9. Samples applied were (A and B) 100 μl, (C) 200 μl of Triton extract, (D) 25 μl human serum in 1 % Triton X-100. Immunoelectrophoresis conditions : 1.5 V/cm for 24 h. Commassie brilliant blue.

Isoelectric focusing of solubilized membrane material in gels containing 1 % Triton X-100 resolves the proteins into numerous bands. Immunoelectrophoresis into antibody-containing agarose gels permits rapid and effective evaluation of the separation obtained. Fig. 1A shows the immunoelectrophoresis pattern obtained using antibodies to human serum proteins. The approximate pH gradient of the electrofocused gel is given. The heaviest immunoprecipitate can be readily identified as serum albumin, and further major precipitates can be identified as C3 and C4 complement components by use of the corresponding monospecific antisera.

Fig. 1B shows the immunoprecipitate obtained when monospecific anti-C9 is incorporated into the agarose gel. A single precipitation arc corresponding to the approximate pH zone 5.8–6.5 is observable. The anodic limb is consistently absent (arrow). Incorporation of anti-C9 plus anti-C5 in the agarose gel (Fig. 1C) results in marked sharpening of the precipitation arc, which decreases in area and also now possesses the anodic, descending limb. Moreover, a second immunoprecipitate is often observed, which fuses with the major precipitation arc. Fig. 1D shows an experiment in which fresh human serum was electrofocused and analysed in the same system. The native C9 component focuses at around pH 4.5–4.6 and yields a clear, symmetrical immunoprecipitate.

These data indicate that, following complement lysis, solubilized membrane material contains C5 and C9 complement components in complex with each other: the complex does not dissociate appreciably during isoelectric focusing in Triton X-100, and migrates to a pH region of around 5.8–6.5.

The two major hemoglobin bands which focus at about pH 6.9 and 7.1 have been found useful markers for the location and comparison of various immunoprecipitates. Thus, the precipitate marked with an arrow in Fig. 1A can readily be correlated to the C9/C5 precipitate of Figs. 1B and 1C. The immunoprecipitate obtained with the complement complex has a typically cloudy appearance with rather diffuse contours.

As can be seen from Fig. 1A, the complement complex can readily be freed from the bulk of contaminating serum proteins simply by sectioning the gels at around pH 6.0–6.5. This corresponds to a gel zone approximately 1.5–1.8 cm beneath the anodic hemoglobin band. We generally include hemoglobin in our preparations because it provides a convenient marker. Crossed immunoelectrophoresis of the concentrated protein sample obtained after electrophoretic elution out of the gel sections yields 7–8 precipitation arcs (Fig. 2), of which two major arcs can be identified

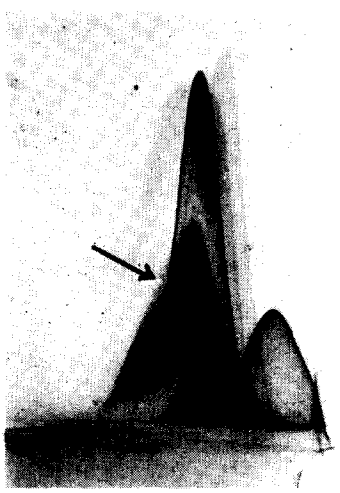

Fig. 2. Crossed immunoelectrophoresis of the protein sample obtained by sectioning gels after electrofocusing. Arrow points to precipitate corresponding to the one obtained with monospecific anti-C9. First dimension electrophoresis : 30 min at 10 $V \cdot cm^{-1}$, second dimension electropohresis : 1.5 $V \cdot cm^{-1}$ for 16 h.

as the C3 and C4 components as described in ref. 26. The cloudy precipitate (arrow) corresponds to the precipitate obtained using monospecific anti-C9, and represents the terminal complement complex.

Sepharose 6B chromatography

Fractions obtained by chromatography of the above sample on Sepharose 6B were directly analysed utilizing fused-rocket immunoelectrophoresis [23]. Fig. 3A shows the precipitation arcs obtained when antibodies against serum proteins were incorporated in the agarose gels. One major component, the bulk of which eluted with or just after the void volume, gave a strong diffuse precipitate with monospecific anti-C9 (Fig. 3B). The breadth of this peak indicates significant size heterogeneity. Four other major precipitates were also found, close together, but clearly separated from the first. Two of these were identified as C3 and C4 components

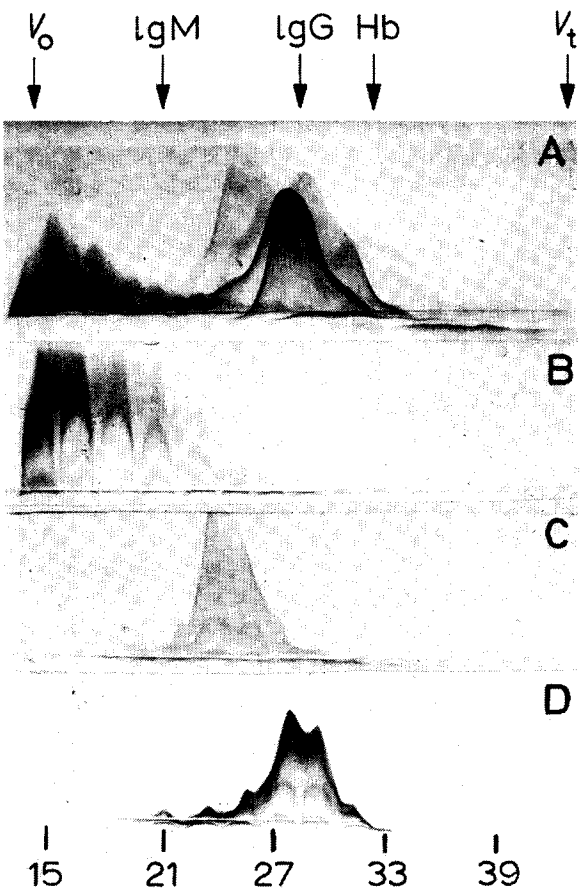

Fig. 3. Fused rocket immunoelectrophoresis of fractions obtained by Sepharose 6B chromatography. Agarose gels contain (A) 15 μl/cm^2 immunoglobulins to human serum proteins, (B) 5 μl/cm^2 monospecific anti-C9, (C) 5 μl/cm^2 monospecific anti-C4, (D) 5 μl/cm^2 monospecific anti-C3. 20-μl samples were applied in A, and 50 μl samples in B–D. Immunoelectrophoresis was performed for 16 h at 1.5V · cm^{-1}. Arrows indicate the void elution volume (V_o), the included (bed) volume (V_t), and the elution positions for IgM, IgC and hemoglobin (Hb). Fraction numbers are shown.

Fig. 4. Crossed immunoelectrophoresis of the purified complement complex analysed with polyvalent immunoglobulins to serum proteins (A, 10 μl/cm^2) and monospecific anti-C9 (B, 5 μl/cm^2). First dimension electrophoresis: 20 min at 10 V · cm^{-1}. Second dimension electrophoresis at 1.5 V · cm^{-1} for 16 h.

(Figs. 3D and 3C, respectively). Very small amounts of these extended into the higher molecular weight regions. Plotting log molecular weight against peak elution volume [27] for IgM, IgG and hemoglobin gave a straight line, on the basis of which the molecular weights of the contaminating C4 and C3 components were estimated to be around 185 000 and 160 000, respectively. The estimated exclusion limit was 4.5 million; this agrees well with the exclusion limit for proteins given by the manufacturer (4 million). The size of the detergent-solubilized complex ranged from 800 000 to 4 million or more, a fraction of which is probably due to bound detergent. Planimetric quantitation of the precipitation arc indicated that approximately 20 % of the precipitated material was smaller than one million daltons, 65 % between one and four million, and 25 % larger than four million.

Crossed immunoelectrophoresis of the pooled, concentrated fractions which precipitated with anti-C9 is shown in Fig. 4. Only one major immunoprecipitate in the α-globulin region of serum proteins was obtained using polyvalent antiserum to human serum proteins (Fig. 4A); this corresponded to the immunoprecipitate obtained with monospecific anti-C9 (Fig. 4B). The precipitation behaviour of the terminal complement complex present in unfractionated Triton extracts with anti-C5, anti-C6 and anti-C9 has been described in detail previously [7], and identical properties were found for the purified material. No immunoprecipitate was obtained using antisera to sheep erythrocytes (Ambozeptor).

These data show that the terminal complement complex can be isolated from target membranes free of other serum proteins with the described, two-step procedure. The time required to complete one preparation is approximately 60 h. 1.5–2.0 mg of the complex can be obtained from 100 ml of human serum in one preparation.

Dodecyl sulfate gel electrophoresis

Fig. 5 shows the electrophoretogram obtained with dodecyl sulfate gel electrophoresis, together with the corresponding scan. Bands are numbered in decreasing order of molecular weight. The molecular weights calculated on the basis of relative mobilities were 190 000, 160 000, 115 000, 93 000, 85 000, 68 000 and 60 000 for bands

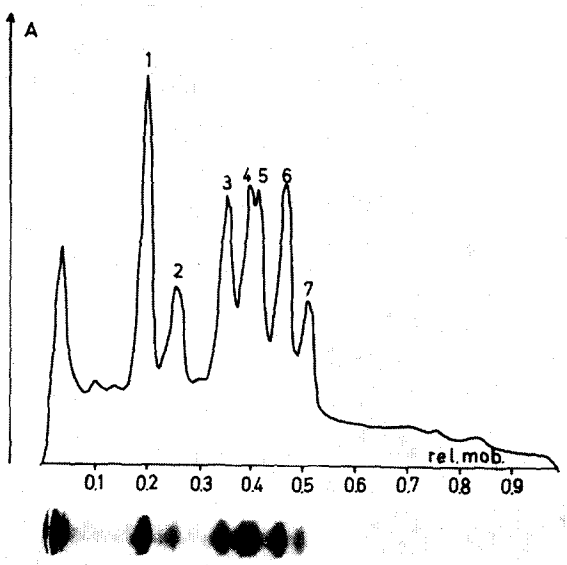

Fig. 5. Dodecyl sulfate gel electrophoresis of the purified complement complex and the corresponding densitometric scan. Bands are numbered in decreasing order of apparent molecular weight. Rel. mob., relative mobility; A, absorbance at 620 nm. Coomassie brilliant blue.

1–7, respectively. An undissociated molecular aggregate was always observed at the gel origin. Molar ratios of approx. 0.7 : 0.3 : 1 : 1 : 1 : 2 : 1 for bands 1–7 were found in five different preparations. All bands stained faintly with periodate-Schiff.

Two-dimensional electrophoretograms yielded information on disulfide bond-

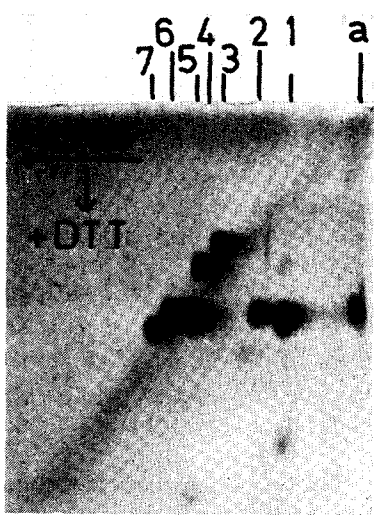

Fig. 6. Two-dimensional dodecyl sulfate gel electrophoresis. First dimension without disulfide cleavage, right to left; second dimension after disulfide cleavage, top to bottom. DTT: dithiothreitol. The positions of bands 1–7 and the molecular aggregate (a) after first dimension electrophoresis are given at the top of the plate.

ing between two or more peptide chains (Fig. 6). The behaviour of bands 1 and 2 upon reduction with dithiothreitol is puzzling; however, both represent proteins possessing subunits linked by disulfide bonds, and the main subunit from each has a molecular weight of approximately 68 000. Band 5 split to yield two subunits of apparent molecular weights around 68 000 and 15 000 upon dithiothreitol treatment. Interestingly, reduction of the molecular aggregate remaining after dodecyl sulfate electrophoresis yielded a major subunit similar in size to the main subunit of bands 1 and 2.

DISCUSSION

Isoelectric focusing and molecular sieving in the presence of nonionic detergents represent powerful tools in membrane protein fractionation. Preservation of antigenic properties of proteins [24, 25, 28] enhances the usefulness of these methods, which can readily be combined with modern analytical immunoelectrophoresis techniques [24, 25]. Electrophoresis of proteins from polyacrylamide gels into agarose, whether after electrofocusing or normal electrophoresis, can present problems due to differences in endosmotic properties of the two media [29–31]. We have found that a thin layer of agarose beneath the polyacrylamide gel allows endosmotic water flow to continue undisturbed across the agarose slab, and resolves all serious problems when gel rods are used in electrofocusing. The system is not as sensitive as the technique of combining thin-layer electrofocusing with immunoelectrophoresis [31], but in our hands gel rods have a higher loading capacity and give more reproducible fractionations of membrane proteins.

Contamination of the final, purified product with significant amounts of sheep erythrocyte membrane proteins is improbable for the following reasons. Firstly, isoelectric focusing followed by second dimension electrophoresis in dodecyl sulfate [12–14] shows that the only sheep erythrocyte membrane proteins present in significant amounts in the pH region 6.0–6.5 after electrofocusing are the high molecular weight "spectrin" bands (Bhakdi, S., unpublished); these are not present in the final dodecyl sulfate gel electrophoretograms (Fig. 5). Secondly, with one exception (a 90 000 dalton protein), no sheep erythrocyte membrane protein in the molecular weight region of any one of the seven bands observed stains with periodate-Schiff [32]. Finally, no clear immunoprecipitate is obtained using antibodies to sheep erythrocytes (Ambozeptor). Consequently, there is little evidence to suggest that any of the seven major bands seen in Fig. 5 represents a membrane protein. This does not exclude very small contaminations with membrane proteins.

It was found that trace amounts of C3 and C4 complement components contaminated some preparations. However, these could be detected only in crossed immunoelectrophoresis and did not result in any change in the dodecyl sulfate gel electrophoretogram. Contamination could best be avoided by sectioning electrofocused gels above pH 6.2 (0.8–1.0 cm below the anodal hemoglobin band), at the cost of lower yields.

The molecular weight of the terminal complement complex formed in a fluid phase has been estimated to be approximately 900 000 [1–6]. We find that the complex prepared from target membranes comprises a heterogeneous population, whose molecular weights range from around 800 000 to about 4 million. This is most probably attributable to the formation of oligomeric aggregates; the possibility that this takes

place during processing of membranes has not been excluded. It is noteworthy that molecular sieving of crude Triton extracts on Sepharose 6B does not separate the terminal complement complex from several contaminants. In particular, large amounts of C3 and C4 components elute in the high molecular weight region. This might be due to the tendency of activated C4 (C4b) to form macromolecular aggregates [33], and to binding of activated C3 to other membrane contituents [19, 34]. Isoelectric focusing is therefore a necessary pre-purification step. Migration of the complex to a pH region 5.8–6.5 is somewhat surprising in view of the known isoelectric points of the native C5-C9 components [35]; most of these lie well below pH 5.8. The isoelectric point of the complex most probably reflects conformational changes in the activated C5-C9 molecules. Evidence for configurational changes in these components after activation and assembly on target membranes has also been documented immunochemically [7].

Although we cannot yet identify the individual protein bands shown in Fig. 5, the marked similarity of our electrophoretogram with the one published by Kolb and Müller-Eberhard [1] for the complex isolated from inulin-activated serum warrants a closer comparison. Table I gives a summary of the data; the following points deserve comment.

Firstly, no aggregation was observed in ref. 1. Secondly, only seven bands can be detected in our preparations, in contrast to eight described in ref. 1. Thirdly, the protein present in greatest relative molar ratio in ref. 1 was C9, having an apparent molecular weight of 76 000; an average of three molecules of C9 were calculated to be attached to each C5 · C8 complex. In contrast, we find one protein (band 6, apparent molecular weight 68 000) to be present in a molar ratio of around 2 : 1 compared to other bands. The two bands of highest molecular weight had a sum molar ratio of one,

TABLE I

SUBUNIT COMPOSITION OF THE TERMINAL COMPLEMENT COMPLEX ISOLATED FROM TARGET MEMBRANES (PRESENT WORK) AND FROM INULIN-ACTIVATED SERUM [1]

Complex isolated from membranes			Complex isolated from inulin-activated serum [1]				Published mol. wt.
Band	Apparent mol. wt.	Molar ratio	Band	Apparent mol. wt.	Molar ratio	Identified as	
1	190 000	0.7	1	170 000	0.8	C5b	185 000 [36, 37]
2	160 000	0.3	2		0.2	C5c	
3	115 000	1.0	3	110 000	1.0	C7	120 000 [38]
4	93 000	1.0	4	99 000	1.28	C6	95 000 [39]
			5	93 000	1.21	C8α	153 000 for C8α+C8β [40]
			6	88 000	3.18	unidentified	
5	85 000	1.0					
			7	76 000	3.21	C9	76 000 [41]
			8	70 000	1.02	C8β	
6	68 000	2.0					
7	60 000	1.0					

which could be explained if they represent C5b and its degradation product, C5c, as shown in ref. 1.

Calibration of gels for molecular weights greater than 150 000 can give rather divergent results, and could account for the different estimates made for the molecular weight of band 1. The molecular weights calculated for bands 3 and 4 in our system correspond well to those given by Kolb and Müller-Eberhard, who identified their bands as C7 and C6, respectively. No certain correlation can be made for the remaining bands, since one band found in the inulin-activated complex is missing in our preparations, and the molecular weights calculated for the other bands do not exactly correspond. On the basis of the molar ratios, however, band 6 probably represents C9; this would imply that bands 5 and 7 represent subunits of C8, and that the unknown component observed in ref. 1 is missing in our preparations. The fact that Kolb and Müller-Eberhard found a high molar ratio for their unknown component (around 3 molecules per complex) further supports this contention, as no protein present in such high relative molar ratio was found in our preparations. The discrepancies in molecular weight found for C8 and C9 could again reflect differences in gel calibration.

Native C5 is known to consist of two peptide chains linked by disulfide bonds [36]. This strengthens the probability that bands 1 and 2 represent C5 complement components, because two-dimensional electrophoretograms show that these bands generate a major subunit of identical size upon disulfide cleavage. We also find that band 5, possibly representing C8α, consists of a large and small peptide chain linked by disulfide bonds. Further studies are necessary to examine whether and how the membrane-bound components differ from their native serum counterparts. It should be stressed that secondary degradation of complement components after assembly on the membranes has not been excluded.

The method described allows isolation of the terminal complement complex for target membranes in a simple, reproducible fashion utilizing commercially available reagents. Studies on its biological and physicochemical properties, and comparison with the complex isolated from inulin-activated serum, may eventually lead to a better understanding of its lytic mechanism.

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