

THE MEMBRANE ATTACK MECHANISM OF COMPLEMENT

VERIFICATION OF A STABLE C5-9 COMPLEX IN FREE SOLUTION*

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We have previously proposed a model for the molecular assembly of the C5-9¹ complex on the surface of cells under attack by complement (1). This model was based on experiments with radiolabeled isolated proteins that allowed quantification of the molar ratios of specifically bound complement molecules. It envisioned a decamolecular complex, in which all five components were tightly bound to each other, having a total mol wt of 995,000 daltons. Since these studies were confined to the cell-bound complex, hydrodynamic measurements could not be performed.

We now want to report the successful attempt to produce a stable C5-9 complex in cell-free solution. This free complex was found to be a by-product of immune cytotoxicity. It further could be induced in the absence of cells by a variety of complement activators. Its composition was found to consist, as predicted, of C5, C6, C7, C8, and C9. The molecular weight closely approximates the anticipated value. The data to be presented substantiate the previously advanced concept.²

Materials and Methods

Human Complement Components.—C3 (2), C5 (2), C6 (3), and C7³ were isolated in highly purified form according to previously published methods. Highly purified C8 and C9 were prepared by modifications of the previously published methods (4, 5), which will be presented in detail elsewhere.⁴ All five proteins were judged free of demonstrable contamination by chromatographic, electrophoretic, and immunochemical criteria. They were trace labeled

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¹ *Abbreviations used in this paper:* Ag-Ab, antigen-antibody; BSA, bovine serum albumin; C5-9 complex, C5b, 6, 7, 8, 9 complex; C7F, cobra venom factor; EA, sensitized sheep erythrocytes; GVB, gelatin containing VB; HS, human serum; HSFa, activated hydrazine-sensitive factor of alternate pathway or C3b; KLH, keyhole limpet hemocyanin; SDS, sodium dodecyl sulfate; VB, Veronal-buffered saline.

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³ Arroyave, C. M., and H. J. Müller-Eberhard. Manuscript in preparation.

⁴ Kolb, W. P., J. A. Haxby, and H. J. Müller-Eberhard. Manuscript in preparation.

with either ^{125}I or ^{131}I by the chloramine-T method of McConahey and Dixon (6). After labeling, each component was dialyzed for 48 h against 4×10 liters of 0.15 M NaCl containing 50 mM chloramphenicol (donated by Parke, Davis and Company, Detroit, Mich.) and 25 μM kanamycin sulfate (Bristol Laboratories, Syracuse, N. Y.). The dialyzed preparations were centrifuged for 2 h at 98,000 g to remove aggregates. Bovine serum albumin (BSA) (Pentex Biochemical, Kankakee, Ill.) was added to a final concentration of 1 mg/ml and the preparations were stored at 0°C . The ^{125}I - and ^{131}I -containing samples were analyzed with a Nuclear-Chicago dual-channel, automatic, well-type gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

Preparation of Sheep Erythrocyte-Antibody-Complement Complexes.—EAC1, 4, $^{oxy}2$ (7), EAC1, 4, $^{oxy}2$, 3 (1), EAC1-7, and EAC1-8 (1) were prepared according to previously published methods.

Complement Activators.—HSFa (C3b) was prepared as described (8). Antigen-antibody (Ag-Ab) complexes were made at fivefold antibody excess by mixing 20 μg of BSA and 10 ml of rabbit anti-BSA (1.6 mg of Ab N_2 /ml). The mixture was incubated at 37°C for 2 h and then at 4°C for 16 h. The antigen-antibody complexes were collected by centrifugation and washed four times with 25 ml of Veronal-buffered saline (VB). The final precipitate was resuspended to 2 ml of VB and the protein concentration determined by the Lowry method (9) using Cohn fraction II as a standard. 2 g of inulin (Pfanstiehl Laboratories, Inc., Waukegan, Ill.) was washed four times with 50 ml of VB and resuspended to 10 ml (200 mg/ml). 2 g of zymosan (Nutritional Biochemical Corp., Cleveland, Ohio) was refluxed in 0.15 M NaCl for 3 h, washed four times with 50 ml of VB, and resuspended to 20 ml (100 mg/ml). Cobra venom factor (CVF) was prepared as described (10). When EA, antigen-antibody complexes, inulin, or zymosan were used as activators, the indicated amounts were placed in 10×75 -mm Falcon plastic tubes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) and centrifuged in a tabletop Sorvall Model GLC-1 centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) for 10 min at 1200 g . The supernatant fluid was removed and freshly drawn human serum (HS), containing the indicated radiolabeled proteins, was added. After incubation at 37°C for 2 h, the reaction mixture was centrifuged and the supernatant subjected to sucrose density gradient analysis, column chromatography, or preparative electrophoresis.

Purification of the C5-9 Complex.—25 ml of freshly drawn HS, containing 200 μg of ^{125}I C8, was incubated with 400 mg of zymosan at 37°C for 90 min with frequent mixing. The zymosan particles were removed by centrifugation at 1200 g and the supernatant was subjected to ascending chromatography on a Bio-Gel A-15M column (5×90 cm) (Bio-Rad Laboratories, Richmond, Calif.). The column had been equilibrated and was eluted with VB containing 1 mg of sodium azide/ml. The flow rate was 15 ml/h and 5-ml fractions were collected. Fractions containing the C5-9 complex, as measured by the incorporated ^{125}I C8 (see Fig. 6), were pooled and concentrated by ultrafiltration to a volume of 10 ml. This pool was rechromatographed on the same column under the same conditions. The ascending portion of the C5-9 peak was pooled and concentrated to 3 ml. The preparation was centrifuged at 1500 g for 30 min and passed through a Millipore filter (0.45 μm pore size) (Millipore Corp., Bedford, Mass.). Chloramphenicol and kanamycin were added to a final concentration of 50 mM and 25 μM , respectively. It was stored at 0°C until used.

Elution of C5-9 Complex from Complement-Lysed EA.—2 ml of iodine-treated serum (11), containing either 10 μg of ^{125}I C9 or 5 μg of ^{125}I C8, was added to a pellet of 10^{10} EA and incubated at 37°C for 3 h with constant mixing. The reaction mixture was then centrifuged in a fixed angle Beckman 40 rotor (Beckman Instruments, Inc., Palo Alto, Calif.) for 2 h at 35,000 rpm. The supernatant was removed and the membrane-containing pellet washed with 4×2 ml of VB. The pellet from EA lysed with ^{125}I C8 containing HS was resuspended in 400 μl of VB and divided into two aliquots that were stored for 120 h at -70°C or 4°C . The pellet from EA lysed with ^{125}I C9 containing HS was resuspended in 200 μl of VB containing 1% sodium dodecyl sulfate (SDS) and held at 37°C for 16 h. After the indicated time, par-

ticulate material was removed by centrifugation at 5000 g for 1 h, and the supernatant subjected to sucrose density gradient analysis.

Sucrose Density Gradient Ultracentrifugation.—10–40% linear sucrose gradients, made with VB, pH 7.5, ionic strength 0.15, were formed in 5-ml cellulose nitrate tubes. The sample size was 100–200 μ l and centrifugation was performed in a Beckman SW 50 or SW 50.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for 16 h at 35,000 rpm and 4°C. Fractions were collected in a dropwise manner (10 drops each) after puncturing the bottom of the tubes.

Determination of the Diffusion Coefficient of the C5-9 Complex.—750 ml of Bio-Gel A-15M (Bio-Rad Laboratories) was equilibrated with VB, containing 1 mg of sodium azide/ml. The gel was deaerated under vacuum for 2 h with frequent mixing and packed in a 2.0 \times 100 cm column (Pharmacia Fine Chemicals, Uppsala, Sweden). 2–3 ml of freshly drawn HS, containing either 2 μ g of [125 I]C9 or 1 μ g of [125 I]C8, was incubated with 40 mg of inulin for 2 h at 37°C with frequent stirring. The inulin was removed by centrifugation and various markers were added to the supernatant before it was analyzed by ascending molecular sieve chromatography. VB was used as eluting buffer and the flow rate was 8 ml/h. 2-ml fractions were collected and assayed for 125 I and 131 I radioactivity, protein distribution, and absorbance at 412 nm to quantitate the hemoglobin marker.

Pevikon Block Electrophoresis.—Small particles were removed from the Pevikon (Mercer Consolidated Corp., Yonkers, N. Y.) by stirring and decanting the supernatant after the large particles had been allowed to settle. This was repeated five times in water. The Pevikon was equilibrated with barbital buffer, pH 8.6, ionic strength 0.05, and poured into a 1 \times 15 \times 50 cm block. The sample consisted of 5 ml of HS diluted 1:1 with VB and 150 μ l containing 40 μ g of purified C5-9 complex having incorporated [125 I]C8. It was applied 7.5 cm from the cathodal end of the block. Electrophoresis was carried out for 36 h at 3 V/cm and 4°C. 1.25-cm segments were cut and eluted twice with 5 ml of saline. All eluates were adjusted to a final volume of 15 ml and analyzed for protein, 125 I content, and C8 and C9 hemolytic activity. The electrophoretic mobility of the C5-9 complex was estimated using a straight line plot of the relative distance traveled by reference proteins on the same Pevikon block vs. their electrophoretic mobility in free solution at an identical pH. The reference proteins and their respective mobilities are: IgG, -1.2×10^{-5} cm² V⁻¹ s⁻¹; transferrin, -3.10×10^{-5} cm² V⁻¹ s⁻¹; albumin, -5.92×10^{-5} cm² V⁻¹ s⁻¹ (12).

Measurement of Competition between C5-9 Complex and EAC1-8 for C9.—EAC1-8 were made by incubating 10 ml of EAC1-7 (1.5×10^8 /ml) with 2 μ g of purified C8 for 30 min at 37°C. The cells were washed four times with 10 ml gelatin containing Veronal-buffered saline (GVB) and standardized to 1.5×10^8 /ml. Increasing amounts of purified C5-9 complex were incubated with 3×10^7 EAC1-8 and 3 μ g of C9 in a total volume of 500 μ l. After 60 min incubation at 37°C the reaction was terminated by the addition of 1 ml of ice cold GVB and centrifuged for 10 min at 800 g. The released oxyhemoglobin was quantitated at 412 nm and the percent of inhibition of EAC1-8 lysis by C5-9 complex was determined.

Binding of [125 I]C9 to Preformed C5-9 Complex.—400 μ l of freshly drawn HS containing 5 μ g of [131 I]C8 was incubated with 10 mg of zymosan at 37°C for 90 min. The zymosan was removed by centrifugation and 200 μ l of the supernatant was stored at 0°C while the remaining 200 μ l was reincubated with 2 μ g of [125 I]C9 at 37°C for 90 min. Both supernatant samples were subjected to sucrose density gradient analysis.

The Reaction Kinetics of Removal of Fluid Phase C9 by C5-9 Complex.—1 μ g of purified C9 was mixed with 25, 125, or 250 μ g of purified C5-9 complex in 1 ml of GVB at 0°C. The reaction mixtures were transferred to 37°C and at various times 50- μ l samples were taken and diluted in 5 ml of ice-cold GVB. All samples were then assayed for residual C9 hemolytic activity. 200 μ l were added to EAC1-8 (3×10^7) and incubated in a total volume of 500 μ l of GVB at 37°C for 45 min.

RESULTS

Formation of the Soluble C5-9 Complex by Activation of the Classic Complement Pathway.—Verification of the C5-9 complex, previously postulated to assemble on a target membrane attacked by complement, depended on the possibility of its occurrence in free solution. As an initial approach we attempted elution of the complex from EA that had been lysed with human serum containing either radiolabeled C8 or C9. After treatment of the washed EA-membranes with SDS or by prolonged storage at 4°C or -70°C, the eluates were examined by sucrose density ultracentrifugation. As seen in Fig. 1,

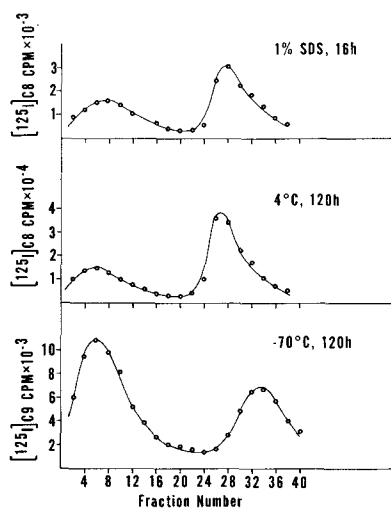


FIG. 1. Ultracentrifugal demonstration of rapidly sedimenting C8 and C9 in eluates from EA lysed by complement (fractions 4-12). Lysis was effected by HS containing [125 I]C8 or [125 I]C9. The washed membranes were incubated with 1% SDS at 37°C for 16 h or stored at either -70°C or 4°C for 120 h. The particulate material was removed by centrifugation and the supernatants were subjected to ultracentrifugation in a 10-40% linear sucrose density gradient at 35,000 rpm for 16 h. Direction of sedimentation was to the left.

each eluate contained fast and slowly sedimenting radiolabeled material. The fast component was indicative of elution of a high molecular weight complex containing C8 and C9.

Next, we examined the possibility of formation of a C5-9 complex in the fluid phase. Samples of human serum containing radiolabeled complement proteins were treated with EA or antigen-antibody precipitates. Examination of treated and untreated serum by ultracentrifugation showed that both activators initiated the formation of heavy, radioactive material. As demonstrated in Fig. 2, the heavy material contained C5, C6, C8, C9, and, not shown in this illustration, also C7. No such heavy material formed when fresh serum

was incubated without complement activators. Consistently more heavy complex was formed when antigen-antibody precipitates were used than when EA served as activator. In the case of EA-induced activation, a significant proportion of the C5-9 complex formed was found to be bound to membrane receptors instead of accumulating in the fluid phase.

Formation of the Soluble C5-9 Complex by Activation of the Alternate Complement Pathway.—Various substances were employed as activators of the al-

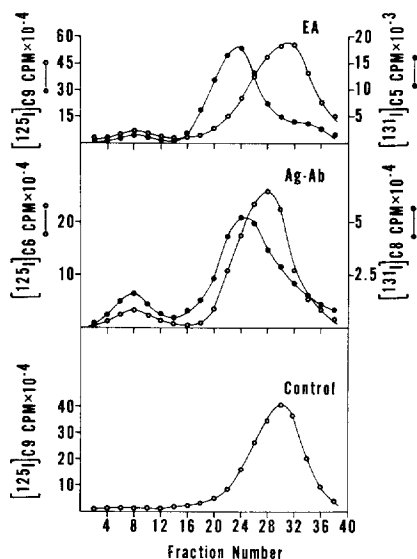


FIG. 2. The formation of a stable and soluble C5-9 complex as a by-product of complement activation by the classical pathway and its demonstration by zone ultracentrifugation. 1.5×10^6 EA or 2 mg of BSA-anti-BSA immune complexes were incubated with $200 \mu\text{l}$ of freshly drawn HS containing the indicated radiolabeled complement proteins for 2 h at 37°C . The EA membranes or antigen-antibody (Ag-Ab) aggregates were removed by centrifugation and the supernatants subjected to sucrose density gradient analysis. The control, which consisted of $200 \mu\text{l}$ of HS containing $[^{125}\text{I}]\text{C9}$, received no complement activator.

ternate pathway. Fig. 3 shows the results obtained with particulate inulin, a polyfructose. C5, C7, C8, C9, and, not shown in this illustration, C6 were incorporated into the rapidly sedimenting radiolabeled component. In contrast, radiolabeled C3 was not detected in the C5-9 complex (Fig. 3, *bottom panel*). In other control experiments, $[^{131}\text{I}]\text{IgG}$ and $[^{131}\text{I}]\text{albumin}$ were also excluded as constituents of the complex.

Table I lists the substances that have been employed in this study as complement activators. Under the conditions used, it is difficult to judge their relative capacity to induce C5-9 complex formation. Nevertheless, a reason-

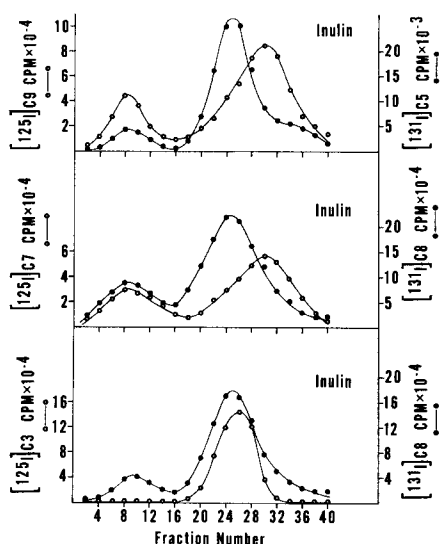


FIG. 3. The formation of a stable and soluble C5-9 complex as a by-product of complement activation by the alternate pathway. 4 mg of inulin was incubated with 200 μ l of freshly drawn HS containing the indicated radiolabeled complement proteins as outlined in the legend to Fig. 2.

TABLE I

Formation of the C5-9 Complex and Consumption of Complement Activities in Human Serum on Treatment with Various Activators

Complement activator	Ability to form fluid phase C5-9 complex	Consumption of hemolytic activity		
		C5	C8	C9
			%	
None	0	0	0	0
EA	+*	60	73	100
HSFa (C3b)	+	10	17	27
Ag-Ab complex	+++	50	42	82
Inulin	++++	80	47	100
Zymosan	++++	100	100	100
CVF	++++	100	38	60

Conditions: 200 μ l of serum were treated for 2 h at 37°C with 1.5×10^9 EA, 100 μ g of HSFa, 2 mg of Ag-Ab, 4 mg of inulin, 2 mg of zymosan, or 25 μ g of CVF.

* Estimated relative size of the 22.4S component.

able correlation is apparent between efficiency of formation of the complex and consumption of C5, C8, and C9.

Physicochemical Properties of the Soluble C5-9 Complex.—Physicochemical measurements were performed on the complex generated in serum by activa-

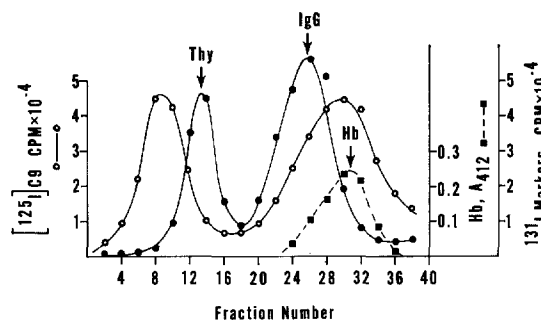


FIG. 4. Sucrose density gradient analysis of the C5-9 complex. Inulin-initiated C5-9 complex formed in HS containing $[^{125}\text{I}]\text{C9}$ was analyzed, as outlined in the legend to Fig. 1, with the following reference proteins: $[^{131}\text{I}]$ thyroglobulin (*Thy*), $[^{131}\text{I}]\text{IgG}$, and hemoglobin (*Hb*).

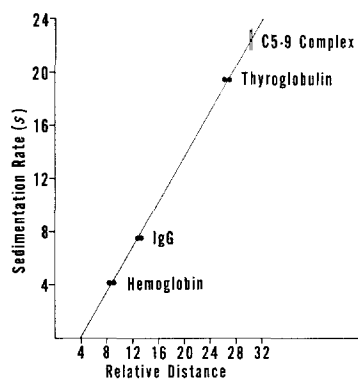


FIG. 5. Determination of the sedimentation coefficient of the C5-9 complex. The data were derived from two separate experiments as outlined in Fig. 4.

tion with inulin. The complex contained either $[^{125}\text{I}]\text{C8}$ or $[^{125}\text{I}]\text{C9}$. The sedimentation coefficient was determined by sucrose density gradient ultracentrifugation (Fig. 4) using hemoglobin (4.5S), IgG (7S), and thyroglobulin (19S) as reference substances. It was found to be 22.5S (Fig. 5). The diffusion coefficient was estimated by gel filtration using Bio-Gel A-15M (Bio-Rad Laboratories) (Fig. 6). Keyhole limpet hemocyanin (KLH), thyroglobulin, IgG, serum albumin, and hemoglobin served as reference substances with known diffusion coefficients. By plotting elution volume vs. the reciprocal of D according to Andrews (13), D of the complex was found to be $1.98 \times 10^{-7} \text{ cm}^2/\text{s}$ (Fig. 7). Assuming a \bar{v} of 0.73, these data allowed calculation of the molecular weight and frictional ratio (Table II).

The electrophoretic mobility of the C5-9 complex was determined at pH 8.6 on Pevikon blocks and was found to be $-4.7 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ (Fig. 8).

Stability of the Soluble C5-9 Complex.—The complex retained its sedimentation and elution characteristics on repeated ultracentrifugation and gel

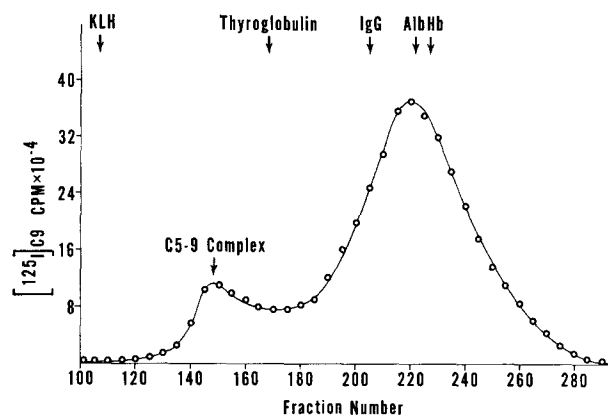


FIG. 6. Molecular sieve chromatography of the C5-9 complex. $[^{125}\text{I}]\text{C9}$ containing C5-9 was subjected to chromatography on Bio-Gel A-15M (2×85 cm column) using $[^{131}\text{I}]\text{KLH}$, $[^{131}\text{I}]\text{thyroglobulin}$, $[^{131}\text{I}]\text{IgG}$, HSA, and hemoglobin as reference proteins. The column was equilibrated and eluted with VB containing 1 mg of sodium azide/ml.

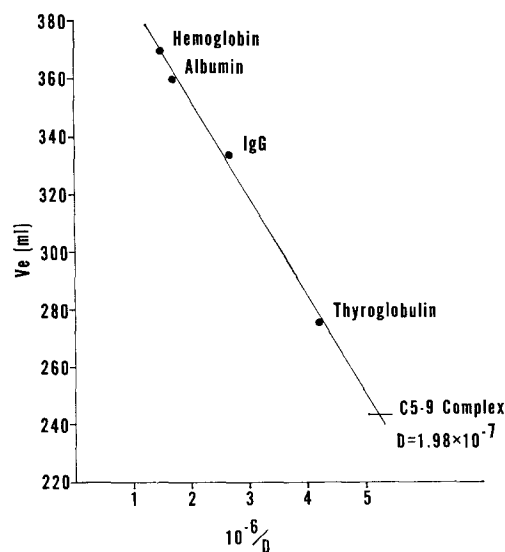


FIG. 7. Determination of the diffusion coefficient of the C5-9 complex. The data for the Andrew plot are derived from experiments illustrated in Fig. 6. The diffusion coefficients of the reference proteins are: thyroglobulin, 2.5×10^{-7} cm²/s; IgG, 3.8×10^{-7} cm²/s; albumin, 6.1×10^{-7} cm²/s; hemoglobin, 6.8×10^{-7} cm²/s.

filtration, respectively. Its size was unchanged after storage for 6 wk at 4°C and pH 7.4. No evidence was obtained for spontaneous dissociation into sub-components. Preliminary results indicate, however, that dissociation can be effected in 0.1% SDS.

TABLE II
Physicochemical Parameters of C5-9 Complex

Sedimentation coefficient	22.5S
Diffusion coefficient	$1.98 \times 10^{-7} \text{ cm}^2/\text{s}$
Mol wt	1.04×10^6
Frictional ratio	1.48
Electrophoretic mobility (pH 8.6)	$-4.7 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$

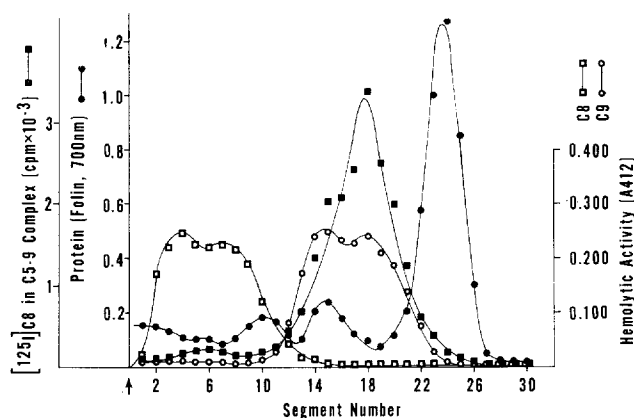


FIG. 8. Electrophoretic behavior of C5-9 complex. ^{125}I C8-radiolabeled C5-9 complex was introduced into HS and analyzed by Pevikon block electrophoresis at pH 8.6. Arrow indicates origin.

Does the Soluble C5-9 Complex Have Hemolytic Activity?—C5-9 complex from sucrose density gradients (Figs. 2 and 3) and gel filtration fractions (Fig. 6) was tested for hemolytic activity using E, EA, and various intermediate complexes of EA and complement proteins. No hemolytic effect was detected even when several thousand C5-9 complexes were offered per cell. In addition, the complex was found not to bind to any of the EA-complement intermediates tested.

Ability of the Soluble C5-9 Complex to Bind Additional C9.—Fig. 9 demonstrates that the C5-9 complex is able to inhibit lysis of EAC1-8 by C9. This finding suggested that the complex was not fully saturated with respect to its C9 binding sites and thus capable of adsorbing additional C9 molecules from the fluid phase. Binding of C9 was directly demonstrated by treating ^{131}I C8 containing C5-9 complex with ^{125}I C9. The treated and untreated samples were then analyzed by zone ultracentrifugation. As seen in Fig. 10, treatment with C9 resulted in binding of a small but definite amount of C9 to the complex. Binding was rapid (Fig. 11) and required a molar excess of complex over free C9. These data indicate that only a small fraction (2-5%) of complexes are capable of adsorbing a small amount of additional C9.

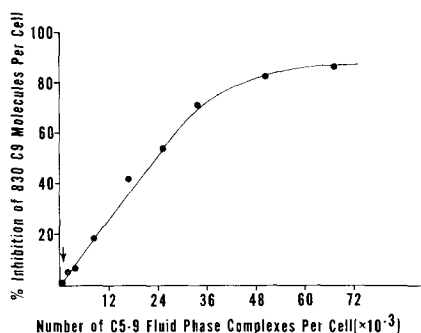


FIG. 9. Competitive inhibition by C5-9 complex of lysis of EAC1-8 by C9. EAC1-8, C9 (830 molecules per cell) and increasing amounts of purified C5-9 complex were incubated at 37°C for 60 min. The arrow indicates the point at which the number of C5-9 complexes offered equals the number of membrane-bound C5-8 sites.

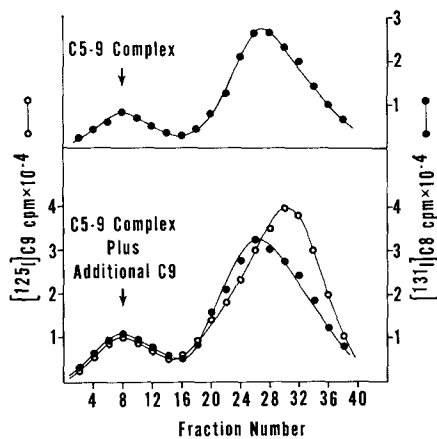


FIG. 10. Binding of additional [^{125}I]C9 to the soluble C5-9 complex. [^{131}I]C8 containing HS was activated with zymosan at 37°C for 90 min. An aliquot of the supernatant was incubated with [^{125}I]C9 at 37°C for an additional 90 min. Both the original and [^{125}I]C9-treated supernatants were subjected to sucrose density gradient analysis as seen in the *top* and *bottom panels*, respectively.

DISCUSSION

The molecular model advanced previously for the membrane attack mechanism of complement was deduced from data concerning the molar ratios and topological relationship of cell-bound complement proteins (1). The objective of the present study was to test the proposed model experimentally. The primary prerequisite for such exploration was the possibility of forming the stable complex in cell-free solution.

The results presented show that a stable, soluble multimolecular complex is generated in whole human serum as a by-product of activation of the classic

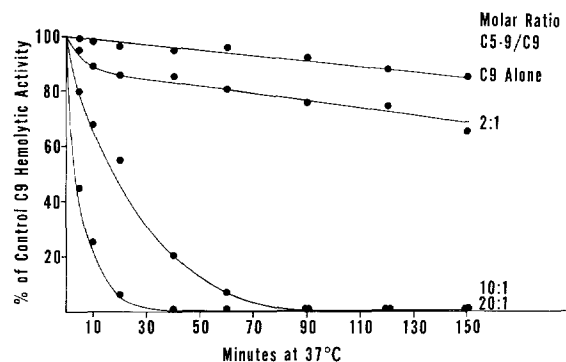


FIG. 11. Kinetic analysis of C9 consumption by soluble C5-9 complex. 1 μ g of C9 was incubated with 0.25, 125, or 250 μ g of highly purified C5-9 complex in 1 ml of GVB at 37°C. Aliquots were withdrawn at the indicated time, diluted 100-fold in 0°C GVB, and assayed for remaining C9 hemolytic activity. The molar ratios of C9 to C5-9 complex were calculated assuming the mol wt of C9 to be 79,000 and that of the C5-9 complex 1.04×10^6 .

and alternate pathways of complement. This complex was shown to incorporate C5 (according to earlier evidence as C5b [14, 15]), C6, C7, C8, and C9. It does not contain C3 or noncomplement proteins such as albumin or IgG. Although its precise composition in quantitative terms is not yet known, the measured mol wt of the soluble complex, 1.04×10^6 , is in excellent agreement with the calculated mol wt of the cell-bound complex, 0.995×10^6 (1). The composition of the cell-bound complex was delineated as containing one molecule each of C5, C6, C7, and C8 and, at saturation of all C9 binding sites, six molecules of C9. The similarity of the molecular weights suggests a similar, if not identical, subcomponent composition for the soluble C5-9 complex.

The soluble complex displayed stability in aqueous solution over extended periods of time, but this stability was not due to covalent bonding of the units: complete dissociation was observed by 0.1% SDS. Its net surface charge was relatively negative considering the charge of the individual subcomponents, all but C9 being β - γ 1-globulins. Nevertheless, the complex behaved as an α -globulin, having the same electrophoretic mobility at pH 8.6 as C9. This observation suggests that a major portion of the surface of the tetramolecular C5-8 complex is covered by C9 molecules (1).

Some observations made by others are pertinent to this study. Thompson and Lachmann (16, 17) reported the occurrence of a stable bimolecular complex of C5 and C6, C5, 6, which formed after activation of C5 by zymosan. The C5, 6, on interaction with C7, was shown to be able to bind to erythrocytes. Although these conclusions were drawn from work with serum reagents, they were confirmed and extended by work with highly purified complement proteins (18, 19). The occurrence of a C5, 6, 7 complex in free solution was postulated by Lachmann and Thompson (17) and by Koethe et al. (20) on the basis of C8 consumption and incorporation of radiolabeled C5 into high-

molecular weight material. Similarly, the occurrence of a C5-8 complex was inferred from the finding of C9 consumption by material excluded on Sephadex G-200 (Pharmacia) (20). Thus, earlier indirect evidence suggested the existence of free C5, 6-, C5, 6, 7-, and C5, 6, 7, 8-complexes.

A sideline of our work, suggested by observations of Koethe et al. (20), was the demonstration that a proportion of the C5-9 complexes are capable of physically binding additional C9. Obviously some C9 binding sites on the C5-9 complex were unsaturated, explaining its inhibitory effect on EAC1-8 lysis by C9.

The following dynamic molecular concept of the membrane attack mechanism of complement is proposed (Fig. 12). In their native form the five proteins of the attack system exhibit affinity for each other (19, 21). They enter into reversible interactions such that C5 associates with C6 and C7, C8 with C5, 6, 7, and C9 with C8 and thus with C5, 6, 7, 8 (22). Formation of the stable C5-9 complex proceeds by self-assembly after enzymatic activation of C5 by the C5 convertases of the classic or alternate pathways. On activation, C5 is cleaved and a low molecular weight fragment (C5a) is dissociated. As a result, the large fragment, C5b, probably undergoes a conformational change allowing adsorptive binding of C6 and C7. The trimolecular complex constitutes the critical molecular arrangement for binding by adsorption of C8 and C9. It is emphasized that the only demonstrable enzymatic reaction in the entire assembling process is that involving C5. All subsequent events appear to be governed by nonenzymatic, physicochemical reactions with a high degree of stereochemical specificity.

Through its C5, 6, 7 portion the complex is enabled to associate itself firmly with receptors of the outer membrane of cells. As a consequence, the mem-

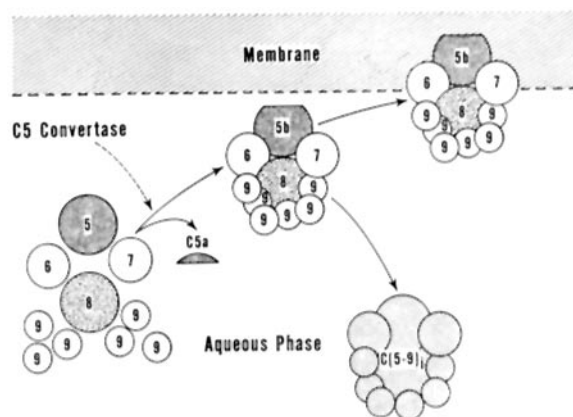


FIG. 12. Schematic representation of formation of a stable C5b-9 complex. On activation of C5, association of components C5b to C9 into a firm complex ensues. This complex has the transient capacity to bind to the surface of a cell and to cause cell lysis. On loss of its binding site the stable arrangement remains in free solution without cytolytic activity.

brane sustains damage and the cell undergoes osmotic lysis. The capacity of the complex to bind to membrane receptors is highly transient and unless binding is achieved within less than 0.1 s (18), the activated binding site is irreversibly lost. The decay product, C(5b, 6, 7, 8, 9)_i, remains as a stable complex in cytolytically inactive form in the fluid phase.

Thus, the nascent C5-9 complex may have two fates with respect to environment and function. The cell-bound complex partially enters the hydrophobic milieu of the membrane interior causing a rearrangement of its microenvironment and thereby functional membrane impairment. The unbound complex remaining in aqueous solution possesses hydrophilic properties and lacks membranolytic functions. Although both forms appear to be decamolecular, they most probably differ in quaternary structure imposed by their disparate environments. Work is underway to determine whether the soluble C5-9 complex has any noncytolytic biologic activity.

SUMMARY

The membrane attack mechanism of complement, C5 to C9, has previously been postulated to associate on the target cell surface to a stable decamolecular complex with a calculated mol wt of 995,000.

A soluble and stable complex consisting of C5, C6, C7, C8, and C9 has now been demonstrated to arise as a consequence of complement activation by the classical or alternate pathway. It has a sedimentation coefficient of 22.5S and a mol wt of 1 million daltons, and it migrates on electrophoresis at pH 8.6 as an α -globulin.

The stable and soluble C5b-9 complex cannot bind to erythrocytes and has no demonstrable cytolytic activity. However, due to partially unsaturated binding sites for C9, it can bind additional C9 and thus function as an inhibitor of lysis of EAC1-8 by C9.

These results support the concept according to which the membrane-bound attack system of complement represents a stable, decamolecular assembly of C5b-9. Unlike its analogue in free solution, the membrane-bound complex is cytolytically active.

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