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Antibody-Complement Complexes

Abstract. *Soluble complexes containing the second and fourth components of guinea pig complement, as well as hemolytic rabbit antibody, have been prepared by elution from sheep erythrocytes carrying these factors. These complexes render erythrocytes susceptible to lysis by the other factors of guinea pig complement, without the usual requirements for hemolytic antibody, Ca++, and Mg++.*

In the present report we describe the preparation and some properties of a new kind of complement (*1–3*) intermediate which hemolyzes unsensitized sheep erythrocytes (*4*) in the presence of EDTA (*5*) if the factors of the C′3 group are supplied. Evidence is presented that this intermediate contains antibody, C′4, and C′2.

For the purpose of this investigation we prepared purified anti-sheep erythrocyte 7*S* antibody possessing low combining affinity (*6*). The 7*S* antibody was separated from rabbit antiserums to boiled sheep-erythrocyte stromata by chromatography on diethylaminoethyl cellulose. The fraction of the antibody population having low combining affinity was then selected by two successive absorptions with washed sheep erythrocytes at 0°C, each followed by elution of the low-affinity antibody fraction at 37°C. The eluted antibody was concentrated by precipitation with ammonium sulfate. The purified antibody was homogeneous as judged by chromatography on Sephadex G-200, analytical ultracentrifugation, and immunoelectrophoretic analysis. Its combination with sheep erythrocytes was completely reversible by dilution, or by shift of temperature. At a cell concentration of 7.7×10^7 per milliliter (1.06×10^5 receptors per cell) and 1.88 μg of antibody per milliliter, 51, 23, and 18 percent of the antibody was bound to the cells at 0°, 30°, and 37°C, respectively.

For preparation of the intermediate SAC′1a, 4, 2a (*7*), 1 ml of packed, washed erythrocyte stromata was treated with 0.5, 1, or 2 mg of purified antibody in a volume ranging from 1 to 10 ml in different preparations. $CaCl_2$ and $MgCl_2$ were added to concentrations of 0.15 and 1 m*M*, respectively. Two milliliters of ice-cold guinea pig serum were added and the reaction was held at 0°C for 10, 20, or 30 minutes. After centrifugal sedimentation, the stromata, presumably in the state StrAC′1a, 4, 2a, or beyond (*8*), were washed once with 0.5 ml of ice-cold M++-buffer (isotonic barbital-NaCl buffer, *p*H 7.3 to 7.4, ionic strength 0.147, containing 0.15 m*M* Ca^{++} and 1 m*M* Mg^{++} as well as 0.1 percent gelatin), and antibody-complement complexes (AC′C) were eluted by incubation for 5 or 10 minutes at 30°C in 15, 20, or 30 ml of M++-buffer containing approximately 500 units of partially purified guinea pig C′2 per milliliter. The inclusion of C′2 in the eluting buffer helped to maintain a steady state of SAC′1a, 4, 2a (*9*); this addition improved the yield, but it is not essential. The stromata were removed by centrifugation at 0°C, and the eluate containing AC′C was stored at −20°C.

The presence of AC′C in such eluates can be demonstrated by lysis of erythrocytes suspended in C′–EDTA (a dilution of guinea pig serum in buffer containing trisodium hydrogen ethylenediaminetetraacetate). Since formation of SAC′4, 2a is blocked under these conditions, the eluate presumably supplies AC′4, 2a, or an intermediate beyond this stage. The C′3 factors are furnished by the guinea pig serum. The possibility that the eluate contains a nonspecific lytic factor can be dismissed because there is no hemolysis without C′–EDTA.

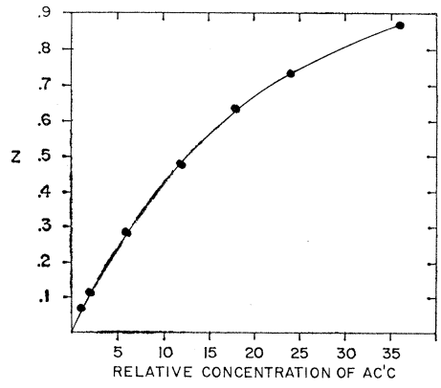

Fig. 1. Dose-response curve of AC′C. The ordinate, "*z*," representing the average number of membrane lesions per erythrocyte, equals the negative $\log_e$ of the fraction of surviving cells. Mixed equal volumes of 1/12.5 C′ in 20 m*M* EDTA buffer and E (3×10^8/ml) in M^{++}-buffer. One-milliliter portions of this mixture were treated with 1.5 ml of AC′C dilution and incubated at 30°C for 90 minutes.

The dose-response curve (Fig. 1) has a shape which fits the properties expected from AC′C, at least qualitatively. In accord with the one-hit theory of complement hemolysis (*10–12*), the curve is entirely concave to the abscissa; that is, its shape is not sigmoidal. The fact that it is not a straight line is to be expected because the antibody in AC′C was selected for low combining affinity, and therefore only part of the AC′C in the reaction mixture would be expected to be in combination with E, the remainder being in the fluid phase. In a companion experiment, not shown here, AC′C prepared with I^{125}-labeled antibody was used; direct proportionality was observed between radioactivity on the cells and the average number of holes produced in the cell membranes after

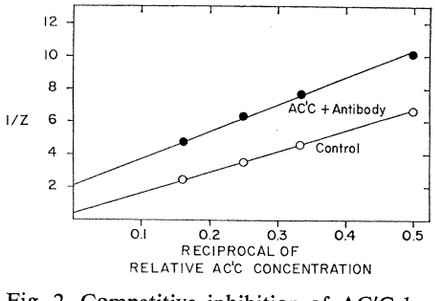

Fig. 2. Competitive inhibition of AC′C by free antibody. Experiment was set up as in Fig. 1, except that AC′C was mixed with purified hemolytic antibody. For control, AC′C was mixed with EDTA buffer.

treatment with C′–EDTA. This shows that the lytic activity of the eluate factor is a direct function of the number of SAC′4, 2a sites which it is capable of generating under the conditions of the experimental test. For analytical purposes it was convenient to make a linear transformation of the response curve in Fig. 1 by plotting $1/z$ against the reciprocal of the relative concentration of eluate (see Fig. 2). Also, for assay of total AC′C activity, in view of the relatively low combining affinity of the antibody, it is necessary to make a graph of $1/z$ plotted against the reciprocal of the erythrocyte concentration and to extrapolate to infinite concentration of cells.

As expected, free hemolytic antibody competes with AC′C and thus inhibits its hemolytic action (Fig. 2). If we were dealing with some nonspecific lytic principle, there would be no a priori reason why purified hemolytic antibody should inhibit its activity. Furthermore, the inhibitory action of hemolytic antibody demonstrates that we are not observing hemolytic action due to a sequential reaction of C′1, C′4, C′2, and so forth; if this were so (despite the EDTA block), addition of hemolytic antibody would enhance activity.

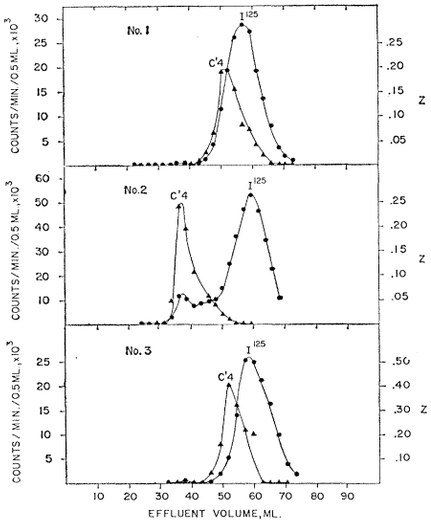

Fig. 3. Chromatography of AC′C on Sephadex G-200: Experiments 1 and 3 were made with a mixture of I^{125}-labeled antibody and partially purified guinea pig C′4. Effluent fractions of 2.2 ml were collected and assayed for radioactivity and C′4 activity. In experiment 2, AC′C prepared with I^{125}-labeled antibody was passed through the column, and effluent fractions were analyzed for radioactivity and C′4 activity. The three experiments were made on the same column, (47.0 by 1.7 cm, at 2° to 4°C, with a flow rate of 8.8 ml per hour).

Since the intermediate SAC′4, 2a decays to SAC′4, with release of C′2a[a] (*12, 13*), it is reasonable to expect similar instability from the postulated AC′4, 2a complex. Indeed, the eluate factor was unstable, and this is why C′2 was incorporated in the eluting buffer so that loss of activity during elution would be prevented by maintenance of the steady state (*9*). Kinetic measurements of the decay at 0°, 30°, and 37°C yielded first-order rate constants of 0.009 min^{-1}, 0.051 min^{-1}, and 0.135 min^{-1}, respectively. These values are reasonably close to those observed with the cellular intermediates (*10*). Also, it is possible to regenerate AC′4, 2a by treatment with C′1a, C′2, Ca^{++} and Mg^{++}, provided erythrocytes are present. The reason for the cell requirement in the regeneration is not clear, but it could be a concentration effect. The decay and regeneration experiments indicate that the active principle in the eluate contains bound C′2a, and in view of recent evidence that C′2a becomes bound to C′4 (*14*), we deduced that the active principle must also contain C′4.

The formation of complex between antibody and C′4 was demonstrated directly by chromatography on a column of Sephadex G-200 (Fig. 3). The first and third experiments were made with a mixture of purified guinea pig C′4 and I^{125}-labeled hemolytic antibody for the purpose of establishing the elution positions of these factors in the free state. In experiment 2, an eluate containing AC′C was passed through the same column. This sample of AC′C had been prepared with I^{125}-labeled hemolytic antibody in order to permit localization of antibody, free or bound, by assay for radioactivity. Two peaks of radioactivity were observed, the major one being eluted in the same position as the free antibody in experiments 1 and 3. The minor, more highly excluded peak of radioactivity presumably represents antibody bound in AC′C. Furthermore, C′4 activity was found in the same, more highly excluded region, indicating that this is C′4 bound to antibody. There was no peak of free C′4. Assays for hemolytic AC′C activity in experiment 2 were unsatisfactory because the fractions were too dilute and the extent of decay too great for precise measurement. Thus, we have presented evidence that hemolytically active complexes containing A, C′4, and C′2 can be eluted from erythrocytes carrying these factors. These complexes may also carry some of the C′3 factors, but no tests for these components were made. Müller-Eberhard and Lepow (*15*) have presented physical evidence of complex formation between γ-globulin and purified human C′4. This observation would be in accord with the concepts and experimental findings presented here. Alternatively, as previously shown (*16*), C′4 can be bound also to cell receptors.

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References and Notes

1. Complement (C′) is an enzymatic system which is found in blood serum and which kills animal and bacterial cells. The C′ system in guinea pig serum comprises nine factors which are designated C′1, C′4, C′2, C′3c, C′3b, C′3e, C′3f, C′3a and C′3d, in their order of action (*2*). In human serum eight factors have been reported, namely, C′1, C′4, C′2, C′3, C′5, C′6, C′7, C′8 (*3*).
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7. S represents a site on the erythrocyte membrane, A is antibody, C′1a is the enzymatically active form of C′1, and 2a refers to that portion of the C′2 molecule which combines with C′4.
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