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The conversion of human complement component C5 into fragment C5b by the alternative-pathway C5 convertase

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The cleavage of human complement component C5 to fragment C5b by the alternative pathway C5 convertase was studied. The alternative-pathway C5 convertase on zymosan can be represented by the empirical formula zymosan–$\overline{C3b_2BbP}$. Both properdin-stabilized C3 and C5 convertase activities decay with a half life of 34 min correlating with the loss of the Bb subunit. The C5 convertase functions in a stepwise fashion: first, C5 binds to C3b and this is followed by cleavage of C5 to C5b. The capacity to bind C3b is a stable feature of component C5, as C5b also has this binding capacity. Component C5, unlike component C3, does not form covalent bonds with zymosan after activation, and C5 is not inhibited by amines. Therefore C5, although similar in structure to C3, does not appear to contain the internal thioester group reported for C3 and C4.

The complement system in blood consists of about twenty proteins that are activated sequentially in response to certain stimuli, such as immune complexes or micro-organisms. Activation of the complement system may occur by two pathways referred to as 'classical' and 'alternative' (for reviews, see Müller-Eberhard & Schreiber, 1980; Reid & Porter, 1981). A central feature in the activation of complement by either pathway is the formation of the proteolytic enzymes C3 convertase and C5 convertase. Although these complex proteinases can be formed in free solution *in vitro*, under physiological conditions they act principally as enzymes bound to surfaces.

The alternative-pathway C5 convertase is a complex proteinase of the complement system that cleaves component C5. Component C5 is a two-chain glycoprotein of mol.wt. 200000, and its activation involves a single proteolytic cleavage on the α-chain (Nilsson & Mapes, 1973; Tack *et al.*, 1979). The alternative-pathway C5 convertase that is generated when zymosan (yeast cell walls) is incubated with serum in the presence of Mg^{2+} has been schematically represented as zymosan–$\overline{C3b_nBb}$ (Medicus *et al.*, 1976*a*). The active site is contained within the Bb subunit (Medicus *et al.*, 1976*b*). The Bb protein is known to have sequence homology with classical serine proteinases (Christie *et al.*, 1980; Mole & Niemann, 1980). Brade *et al.* (1973) and Medicus *et al.* (1976*a*) have proposed that the alternative-pathway C3 and C5 convertases are distinct entities.

Vogt *et al.* (1978) have proposed that the binding of component C5 to surface-bound C3b generated an altered conformation of component C5 that was cleavable by either the alternative-pathway or classical-pathway convertases. Fluid-phase alternative-pathway C5 convertase has not been observed (Medicus *et al.*, 1976*a*; Vogt *et al.*, 1978) except under special conditions of low ionic strength and high C3b concentration (Isenman *et al.*, 1980). It was further proposed that component C5 may bind C3b in a multivalent fashion (Isenman *et al.*, 1980).

The objective of the present work is to explore further the conversion of component C5 to C5b by the alternative-pathway C5 convertase. In addition, as component C3 has been proposed to contain a reactive thioester group (Janatova *et al.*, 1980*a*; Sim *et al.*, 1981; Tack *et al.*, 1980), the free thiol content of component C5 and C5b was investigated along with the binding properties of C5b.

Abbreviations used: SDS, sodium dodecyl sulphate; EAC143 cells, sheep erythrocytes bearing antibody and complement components $C\bar{1}$, C4b and C3b; *K*, the equilibrium association constant. The nomenclature of complement components and subcomponents is that recommended by the World Health Organisation (1968). DGVB^{+2}, 140 mM-glucose/71 mM-NaCl/2.5 mM-sodium 5,5-diethylbarbiturate (pH 7.5)/0.1% gelatin/0.5 mM-$MgCl_2$/0.15 mM-$CaCl_2$.

Methods and materials

Materials

Phenylhydrazine hydrochloride was a product of

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May and Baker Ltd., Dagenham, Essex, U.K. Hydrazine sulphate was from Hopkin and Williams, Chadwell Heath, Essex, U.K. Salicylhydroxamic acid was purchased from Aldrich Chemical Co., Gillingham, Dorset, U.K. Hydroxylamine hydrochloride was from Cambrian Chemicals, Croydon, Surrey, U.K. Sheep erythrocytes were obtained from Tissue Culture Services, Slough, Berks., U.K. *p*-Chloromercuri[^{14}C]benzoate was from CEA, Gif-sur-Yvette, France. Iodo[^{14}C]acetamide was a product of Amersham Radiochemicals, Amersham, Bucks., U.K. All other materials and proteins that were employed for these experiments were obtained or purified as in the preceding paper (DiScipio, 1981).

Haemolytic assays

Component C3 was assayed with EAC14 cells by the method of Tack & Prahl (1976), KBr-treated serum being used as a source of C3-deficient serum. Component C5 was assayed by utilizing EAC14 cells with a C5-deficient plasma that was made by passage of 50ml of $BaCl_2$-treated human plasma containing 10mM-EDTA over a column (2.5cm × 10cm) of rabbit anti-(human C5) antibody covalently bound to Sepharose. The gelatin/veronal buffers that were used for the assays were made as described by Nelson *et al.* (1966).

Estimate of the decay rates of C5 and C3 convertase

To 12mg of zymosan–C3b prepared as described in the preceding paper (DiScipio, 1981) was added 14μg of properdin, 50μg of ^{125}I-labelled factor B (3×10^6c.p.m.), 0.5μg of factor D in 10mM-imidazole/HCl buffer (pH7.3)/0.15M-NaCl/3M-$MgCl_2$. After 20min at 37°C, 80μl of 0.2M-EDTA was added and the zymosan was centrifuged at 9980*g* for 1min. The supernatant was discarded, and the pellet was washed once with 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl and transferred to another tube, which was centrifuged at 9980*g* for 1min. The pellet was resuspended in 400μl of 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl. This activated zymosan was kept at 37°C with intermittent mixing, and, as a function of time, portions were withdrawn and assayed for radioactivity and C3 and C5 convertase activities. Controls were performed by adopting the same procedure but with zymosan devoid of covalently associated C3b. At various time intervals, 25μl of the activated zymosan mixture was layered over 250μl of oil (Lenzol) in a plastic tube; after centrifugation at 9980*g* for 1min to separate the material bound to the solid phase from the aqueous phase (Sim, E. & Sim, R. B., 1981) the bottom of the tube containing the pellet was cut off and counted for ^{125}I radioactivity. For C3 convertase activity assays, 25μl of the activated zymosan mixture was withdrawn at various time periods and centrifuged at 8000*g* for 2min in a silicone-treated glass tube; the supernatant was discarded, and 100μl of a 1.4mg/ml solution of native C3 in 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl was added. The reaction was allowed to proceed at 37°C for 30min with intermittent mixing; then the C3 activity was assayed as described above. For C5 convertase activity, 25μl of the activated zymosan mixture was removed at various time intervals and centrifuged at 8000*g* for 2min in a silicone-treated glass tube. The supernatant was discarded and 50μl of a 0.35mg/ml solution of component C5 in 10mM-imidazole/HCl (pH7.3)/0.075M-NaCl was mixed with the pellet, and the reaction was allowed to proceed at 37°C for 90min, after which the C5 activity was assayed as previously described.

Influence of native C3 and C3b on zymosan-associated C5 convertase activity

To 17mg of zymosan–C3b was added 18μg of properdin, 140μg of factor B, 0.8μg of factor D in a final volume of 600μl in 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl/3mM-$MgCl_2$ and the mixture was incubated for 20min at 37°C. Then 60μl of 0.2M-EDTA was added and the mixture was centrifuged at 9980*g* for 1min. The supernatant was discarded. The mixture was resuspended in 600μl of 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl and 150μl portions were added to separate silicone-treated glass tubes. These were centrifuged at 8000*g* for 2min, and the supernatant was discarded. The activated zymosan was mixed with 50μg of C5 or with 50μg of C5 with 500μg of C3 or with 50μg of C5 with 500μg of C3b. The final volume of each reaction was 300μl, and the buffer was 10mM-imidazole/HCl (pH7.3)/0.1M-NaCl. Samples were allowed to react at 37°C and, at various times, 25μl portions were removed, centrifuged, and assayed for component C5 activity as described above.

Influence of C5 on the zymosan-associated C3 convertase activity

The same procedure was performed as described above for the generation of a zymosan-associated C3/C5 convertase. To the activated zymosan was added 200μg of C3 or 200μg of C3 with 15μg of C5. The final volume of each reaction was 150μl and the buffer was 10mM-imidazole/HCl (pH7.3)/0.1M-NaCl. At various times component C3 activity was assayed.

Conversion of component C5 into C5b and the binding of 'nascent' C5b to zymosan–C3b

Zymosan (3.8mg) bearing 25pmol of covalently bound C3b was incubated with 10μg of factor B, 4μg of properdin, 0.05μg of factor D in a final volume of 150μl of 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl/3mM-$MgCl_2$ in a silicone-

treated glass tube. After 20min at 37°C, 20μl of 0.2M-EDTA and 150μl of water were added. The activated zymosan was centrifuged at 8000g for 2min and the supernatant was discarded. To the pellet, 250μl of ^{125}I-labelled C5 (0.25mg/ml; 225000c.p.m.) was added. At various times, 30μl portions were withdrawn and layered over 250μl of oil (Lenzol). Each assay tube was centrifuged at 9980g for 1min. The bottom of the tube was cut off and counted for ^{125}I radioactivity. Portions of the supernatant were counted for radioactivity and assayed for C5 haemolytic activity. Controls were performed with untreated zymosan mixed with ^{125}I-labelled C5. Control values were subtracted from the experimental determinations.

EAC143 production and binding studies

To 6ml of 6×10^9 EAC14 cells in $DGVB^{+2}$ there was added 1ml of a 1.3mg/ml solution of C3 and 40μg of component C2 in the same buffer. After 1h at 37°C the cells were washed five times in 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl and five times with $DGVB^{+2}$. Determinations of the amount of C3b bound to the cells were achieved by running a parallel experiment with identical conditions except that ^{125}I-labelled C3 was employed. An estimate of non-specifically bound C3 was made by incubating EAC14 cells with ^{125}I-labelled C3 in the absence of C2. The value for the non-specifically bound radioactivity was subtracted from the experimental radioactivity, thus providing an estimate for the amount of specifically bound ^{125}I-labelled C3b.

Studies of the binding of component C5 to EAC143 cells were performed as described in the preceding paper (DiScipio, 1981) for zymosan-C3b, except that the buffer was 10mM-imidazole/HCl (pH7.3)/0.075M-NaCl/0.3% glucose. Controls were performed with EAC14 cells.

Reaction of component C5 with amines

Samples of component C5 (44μg) in 10mM-imidazole/HCl (pH7.3)/0.075M-NaCl were mixed with various amounts of hydrazine, hydroxylamine, phenylhydrazine, and salicylhydroxamic acid for 1.5h at 37°C in a final volume of 250μl. After the samples were dialysed three times against 1000vol. of 10mM-imidazole/HCl (pH7.3)/0.15M-NaCl, they were assayed for C5 activity as described above.

Denaturation of component C5 by KSCN

To samples of component C5 (30μg) in 10mM-imidazole/HCl (pH7.3)/0.1M-NaCl, there was added 5M-KSCN in portions from 0 to 25μl in a final volume of 120μl with or without 10mM-hydrazine, and the samples were incubated at 37°C for 1.5h. These samples were desalted on a 2ml column of Sephadex G-25 in the same buffer and the C5 activity was assayed as described above.

Results

SDS/polyacrylamide-gel electrophoresis of component C5 and C5b

SDS/polyacrylamide gels of human complement component C5 and C5b are shown in Fig. 1. As noted by others (Nilsson & Mapes, 1973), component C5 is activated by C5b by a single proteolytic cleavage of the α-chain (mol.wt. 117000) to give rise to a new α'-chain (mol.wt. 106000) and an activation peptide (mol.wt. 11000). The β-chain (mol.wt. 85000) remains unchanged in the conversion. Component C5 loses all assayable haemolytic activity by the activation process. Fragment

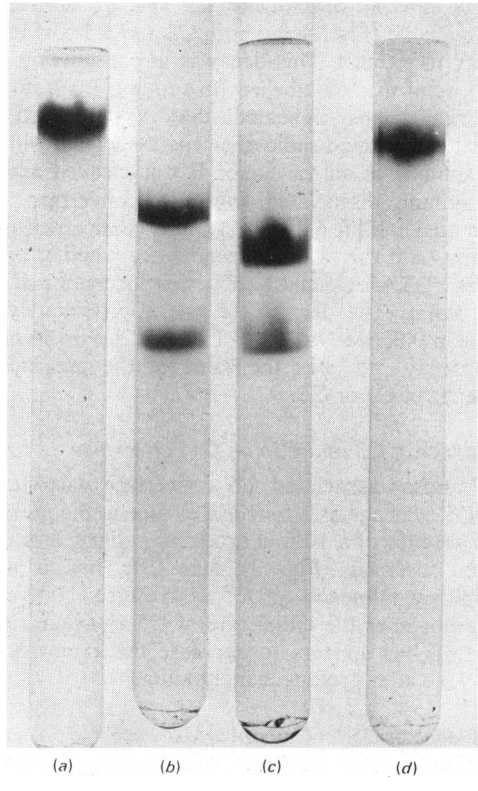

Fig. 1. *SDS/polyacrylamide gels of human complement component C5 and C5b*

The identity of the gels is as follows: (*a*) unreduced C5; (*b*) reduced C5; (*c*) reduced C5b; (*d*) unreduced C5b. Protein samples (20–30μg) were run on 5.8% polyacrylamide gels for 3.5h at 60mA, and the gels were stained for protein with Coomassie Brilliant Blue R. The method used was that of Weber & Osborn (1969) as modified by Kisiel *et al.* (1976).

C5b has no haemolytic activity when assayed with EAC14 or EAC143 cells.

Formation and decay of C5 convertase

A fluid-phase C5 convertase was not observed under the conditions used in these studies, in agreement with Medicus *et al.* (1976*a*) and Vogt *et al.* (1978). C5 convertase could not be generated from zymosan bearing non-covalently associated C3b, but an alternative-pathway C3 convertase can be formed from fluid-phase C3b or zymosan bearing non-covalently bound C3b. Only zymosan bearing C3b bound by its labile binding site was effective in the formation of C5 convertase. The capacity to form C5 convertase on zymosan was a function of the amount of bound C3b, but the capacity to form C5 convertase was independent of conditions of ionic strength between 0.03 and 0.25 M-NaCl, and pH between pH 5.0 and 9.0 under which conditions the C3b was deposited on the zymosan.

The alternative-pathway C3 and C5 convertase are unstable (Fig. 2) and both C3 and C5 convertase decay in parallel. This decay is in conjunction with the loss of the ^{125}I-labelled Bb subunit. Inspection of autoradiograms indicated that virtually all the specifically bound radiolabel was associated with the Bb subunit as all the factor B was cleaved and the Ba subunit dissociated from the convertase. The estimated half-life of both C3 and C5 convertases was 34 min, and this was independent of whether or not 5 mM-EDTA or 5 mM-$MgCl_2$ was incorporated into the buffer, The decay rate was approximately the same in 0.075 M-NaCl or 0.15 M-NaCl. Furthermore, the decay rate was the same in the presence or absence of C3 or C5.

Influence of C3 and C3b on C5 convertase

Zymosan-associated C5 convertase was studied for C5 cleavage as a function of time in the presence and absence of a 10-fold excess of native C3 or C3b. It is observed (Fig. 3) that C3b has a small inhibitory influence on C5 convertase. Native C3 does not alter the initial rate of C5 cleavage (up to 90 min), but appears to decrease the extent of C5 cleavage after prolonged incubation.

Influence of C5 on C3 convertase

The influence of C5 on the cleavage of C3 by zymosan-associated C3/C5 convertase was studied as a function of time. It was observed (Fig. 4) that C5 could markedly inhibit C3 activation even when C3 is in 13-fold molar excess. Inhibition of a fluid-phase alternative-pathway C3 convertase by C5 was not observed.

Binding of 'nascent' C5b to zymosan

A time course of the activation of ^{125}I-labelled C5 is shown in Fig. 5. Under the conditions used, it is observed that, as C5 is converted into C5b on the zymosan, about 50% of the C5b binds to the zymosan. The amount of C5b bound after 180 min exceeds by 6-fold the amount of C3b on the zymosan; however, none of the ^{125}I-labelled C5b appears to be covalently bound, since five washings with 10 mM-imidazole/HCl buffer (pH 7.3)/0.65 M-NaCl/0.3% gelatin removes 92% of the ^{125}I-labelled C5b. Further high-salt washings remove only negligible amounts of the remaining ^{125}I-labelled C5b,

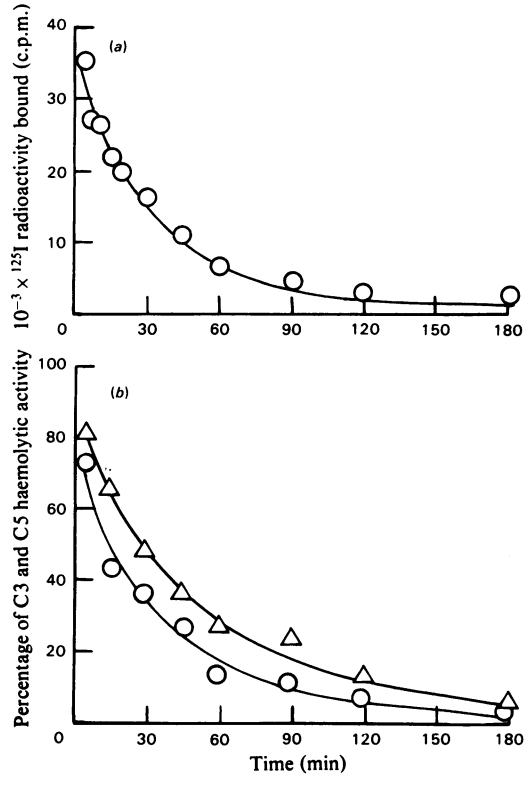

Fig. 2. *Decay of the zymosan-associated alternative-pathway C3 and C5 convertases*

(*a*) The release of the ^{125}I-labelled Bb subunit as a function of time at 37°C. Portions (25 μl) of a zymosan–C3/C5 convertase mixture were layered over 250 μl of oil (Lenzol) and centrifuged at 9980 *g* for 1 min. The pellet and a portion of the supernatant was counted for radioactivity. (*b*) The decay of C3 and C5 convertase activities as a function of time. At various times, 25 μl portions of an incubation mixture of zymosan–C3/C5 convertase were withdrawn and assayed for C3 (○) or C5 (△) convertase activity according to the Methods and materials section.

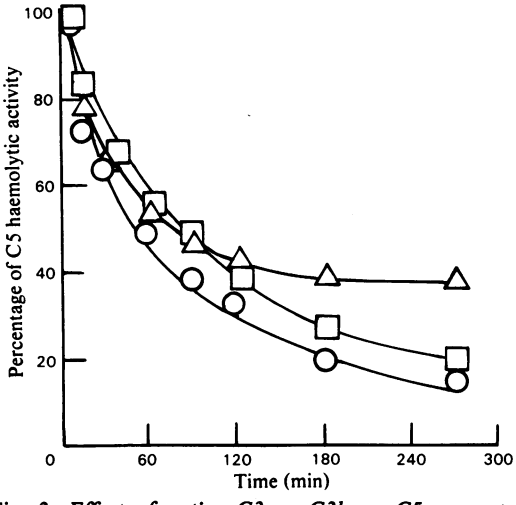

Fig. 3. *Effect of native C3 or C3b on C5 convertase activity*

Component C5 (50 μg) was made to react with zymosan–C3/C5 convertase in the absence (O) or presence of 500 μg of native C3 (△) or C3b (□). The C5 haemolytic activity was assayed as a function of time.

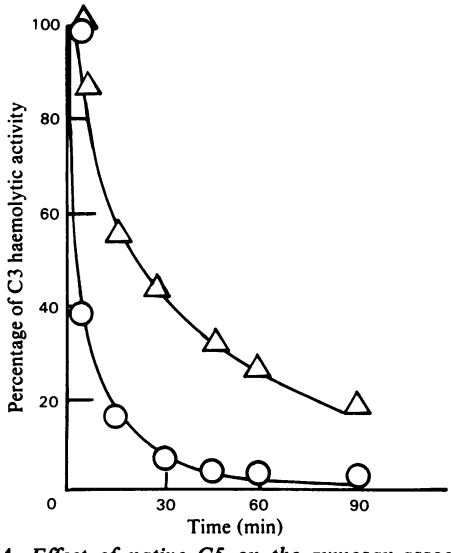

Fig. 4. *Effect of native C5 on the zymosan-associated C3 convertase activity*

Component C3 (200 μg) was made to react with zymosan–C3/C5 convertase in the absence (O) or presence of 15 μg of C5 (△). The C3 haemolytic activity was assayed as a function of time.

but all of it is removed by incubation at 37°C for 1 h and washing with 10 mM-Tris/H_3PO_4 (pH 7.0)/0.5% SDS/6 M-urea.

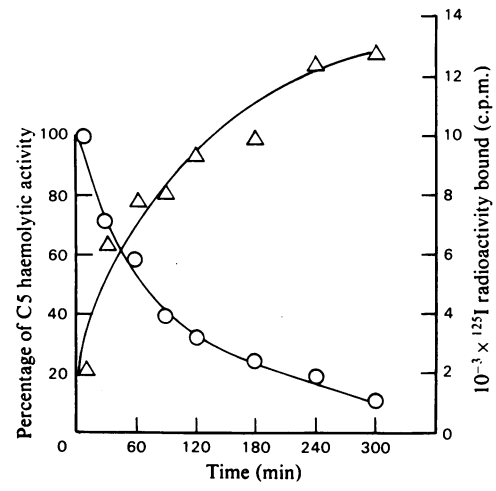

Fig. 5. *The activation of component C5 by a zymosan-associated C5 convertase and the binding of 'nascent' C5b to the zymosan*

Radiolabelled component C5 (60 μg) in 250 μl was added to a zymosan–C5 convertase in 10 mM-imidazole/HCl, pH 7.3/0.075 M-NaCl, and at various times, 30 μl portions (27000 c.p.m.) were withdrawn and layered over 250 μl of oil (Lenzol). The radioactivity in the pellet (△) and the C5 haemolytic activity in the supernatant (O) were determined.

Binding of components C5 and C5b to zymosan–C3b and EAC143 cells

The results of the binding of 'nascent' C5b to zymosan–C3b made it worthwhile comparing the binding of fluid-phase C5 and fluid-phase C5b to zymosan-C3b and EAC143 cells. It was observed (Fig. 6) that component C5 has a K value of $1.1 \times 10^7 M^{-1}$ and a C5/C3b ratio of 1.1 for zymosan-bound C3b, and a K value of $5.1 \times 10^6 M^{-1}$ with a C5/C3b ratio of 1.0 for EAC143 (in 0.075 M-NaCl). Fragment C5b has a K value of $9.4 \times 10^6 M^{-1}$ and a C5b/C3b ratio of 0.9 for zymosan–C3b, and a K value of $3.3 \times 10^6 M^{-1}$ with a C5b/C3b ratio of 1.0 for EAC143 (in 0.075 M-NaCl). Furthermore, it was observed that component C5 and C5b can compete with each other for binding to EAC143. Thus both component C5 and C5b have a stable binding site for bound C3b.

Free thiol content of C5

The reactions of C5 and C5b with *p*-chloromercuri[^{14}C]benzoate results in the incorporation of 1.1 and 1.0 mol of reagent per mol of protein respectively. The reaction of C5 and C5b with iodo[^{14}C]acetamide results in the incorporation of 1.1

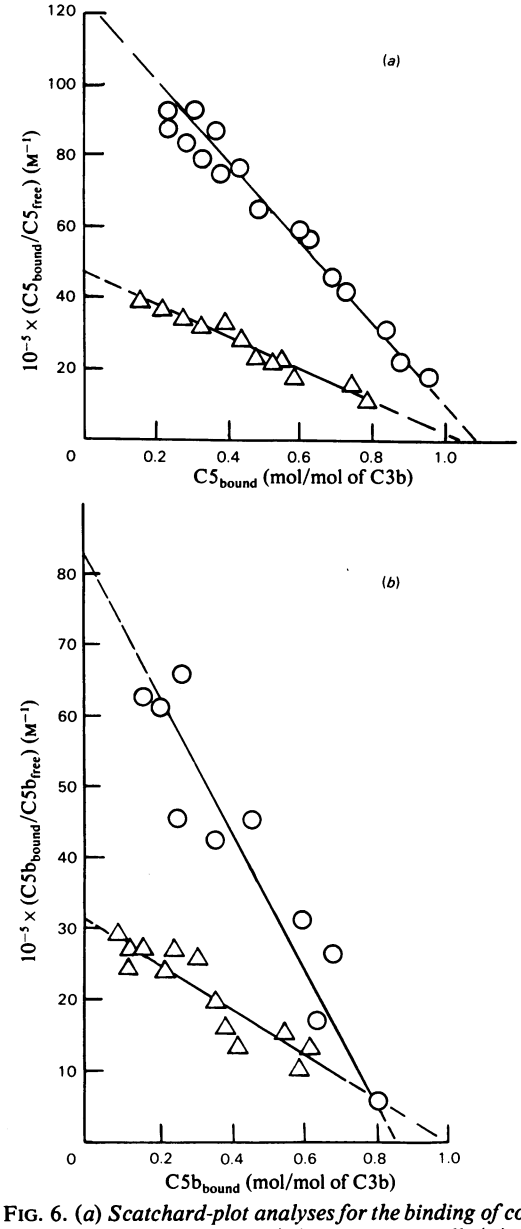

FIG. 6. *(a) Scatchard-plot analyses for the binding of component C5 to zymosan–C3b* (○) *or EAC143 cells* (△) *and (b) Scatchard-plot analyses for the binding of C5b to zymosan–C3b* (○) *or EAC143 cells* (△)

For the binding studies with zymosan–C3b the buffer was 10mM-imidazole/HCl (pH 7.3)/0.075M-NaCl/0.3% (w/v) gelatin. For the binding studies with EAC143 cells the buffer was the same as above, but it also contained 2.3% (w/v) glucose.

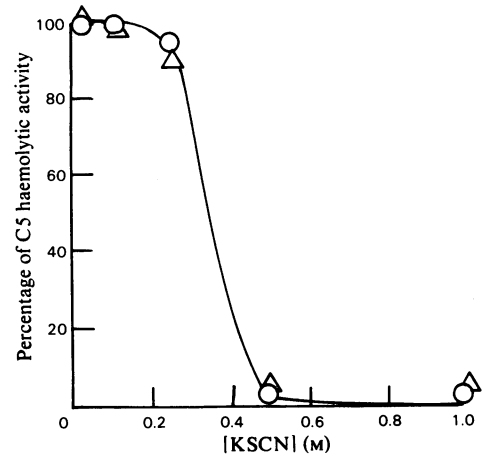

Fig. 7. *Denaturation–inactivation of component C5 as function of KSCN concentration in the absence* (○) *or presence* (△) *of 10mM-hydrazine*

and 0.7mol of reagent per mol of protein respectively. In non-denaturating buffers [10mM-Mops (4-morpholine-ethanesulphonic acid) (pH 6.0)/0.35M-NaCl for the *p*-chloromercuribenzoate reaction or 10mM-Tris/HCl (pH 8.6)/0.35M-NaCl/1mM-EDTA for the iodoacetamide reaction] the thiol reagents still react with native C5 and C5b, but the rate of incorporation is slower than in the buffers containing 4–6.5M-guanidine. In contrast with the case of component C3 (Janatova *et al.*, 1980*a*), an additional free thiol group was not observed to become exposed in C5b after activation. The reaction of native C5 with *p*-chloromercuribenzoate did not inactivate this protein.

Effect of amines on component C5 activity

As components C3, C4 and α_2-macroglobulin are inactivated by amines, it was of value to explore the possibility of component C5 being amine-sensitive. Component C5 was made to react with amine compounds as described in the Methods and materials section. The results were that 100mM-hydrazine, 100mM-hydroxylamine, 100mM-phenylhydrazine, and 100mM-salicylhydroxamic acid all failed to inactivate component C5. Furthermore, 10mM-hydrazine does not potentiate the denaturation–inactivation of component C5 by KSCN (Fig. 7).

Discussion

This present work has dealt with the conversion of human complement component C5 into C5b. It was previously established that this process involves a single cleavage of the α-chain of component C5 with the formation of the anaphylatoxin, C5a (Fernandez & Hugli, 1977; Nilsson & Mapes, 1973).

The observed half-life of 34min (Fig. 2) at 37°C for both C3 and C5 convertases indicates that the

association of B6 with C3b is of a similar nature for both C3 and C5 converase.

As I have observed an apparent univalent binding of component C5, factor B and properdin to C3b bound to zymosan [the preceding paper (DiScipio, 1981)], the simplest empirical formula that may represent the alternative-pathway C5 convertase is zymosan–$\overline{C3b_2BbP}$. One molecule of C3b is associated with the Bb subunit and properdin, and one molecule of C3b serves to bind component C5.

It is suggested that the alternative-pathway C5 convertase operates in stepwise fashion: first, the C5 binds to covalently bound C3b (Vogt *et al.*, 1978), and this is followed by cleavage by the Bb subunit. Results of studies on the rate of C5 cleavage in the presence of C3b and C3 (Fig. 3) and of the rate of C3 cleavage in the presence of C5 (Fig. 4) are consistent with a stepwise mechanism of C5 convertase.

It is necessary for the C3b molecules of C5 convertase to be in proximity and perhaps correct orientation for C5 cleavage, and this can be accounted for by the fact that the C3b molecules deposited on zymosan are in dense clusters of about 30 C3b molecules per cluster [see the preceding paper (DiScipio, 1981)].

When C5 is activated on zymosan in 0.075 M-NaCl, about 50% of the C5b formed was observed to bind to the zymosan under the conditions employed (Fig. 5). The amount of C5b bound exceeded by 6-fold the amount of C3b on the zymosan. However, when fluid-phase C5 or C5b was employed for binding with zymosan–C3b or EAC143, the maximum number of molecules of C5 or C5b bound per molecule of C3b was one (Fig. 6); saturation binding was always observed. Thus 'nascent' C5b is endowed with enhanced binding capacity; however, all of the bound C5b can be removed by washing with 0.5% SDS/6 M-urea/10 mM-Tris/H_3PO_4 buffer, pH 7.0. Thus it is unlikely that 'nascent' C5b can associate with the zymosan by a covalent bond, as has been proposed for 'nascent' C3b and 'nascent' C4b (Law & Levine, 1977; Law *et al.*, 1979; Law *et al.*, 1980; Campbell *et al.*, 1980). In addition, findings by Law *et al.* (1980) have shown that convertase-deposited C5b on erythrocytes did not show the C5b to be covalently associated with any high-molecular-weight entities as was observed for C3b and C4b.

In contrast with components C4, C3 and the proteinase inhibitor α_2-macroglobulin (Janatova *et al.*, 1980*b*; Salveson & Barrett, 1980; Harpel *et al.*, 1979, Sim & Sim, 1981), component C5 does not undergo a denaturation-induced autolytic cleavage (Sim & Sim, 1981). Furthermore, the studies presented here indicate that, in contrast with components C3, C4 and α_2-macroglobulin (Ratnoff *et al.*, 1954; Müller-Eberhard & Biro, 1963; von Zabern *et al.*, 1980; Harpel & Hayes, 1979), component C5 is neither inhibited by amines nor does 10 mM-hydrazine potentiate the denaturation of component C5 by KSCN (Fig. 7). Thus it is unlikely that component C5 contains an active centre that is chemically similar to component C3, component C4 and α_2-macroglobulin. For these proteins the active centre has been proposed to be a thioester (Janatova *et al.*, 1980*a*; Tack *et al.*, 1980; Sim *et al.*, 1981; Sottrup-Jensen *et al.*, 1980).

Component C5 has a stable binding site for covalently bound C3b, and C5b also has this binding capacity (Fig. 6). Both C5 and C5b bind to C3b as a 1:1 association with a similar binding affinity. The affinity of C5 or C5b for C3b was 2–3-fold lower when the C3b was associated with erythrocytes rather than zymosan. This may be because C3b on the cell surface is less accessible than C3b on zymosan, perhaps as a result of negative charges on the cell surface. As it has been observed that C3b on erythrocytes can potentiate the action of the $\overline{C5b6}$ complex (Hammer *et al.*, 1976; Goldlust *et al.*, 1974), it is highly probable that the $\overline{C5b6}$ complex can bind to the cell-associated C3b by the C5b subunit.

As with C3b and inactivated C3, component C5 and C5b contain a single free thiol group on the α-chain and α′-chain respectively. The functional significance of this free thiol group has not been established by the present studies, but this group does not become exposed when C5 is converted into C5b, in contrast with the case of C3 (Janatova *et al.*, 1980*a*). Treatment of native C5 with *p*-chloromercuribenzoate did not inhibit the haemolytic activity of this protein. These results are not in accord with those obtained in previous work (Polley & Müller-Eberhard, 1969), where two free thiol groups were reported to exist in component C5. The 'free' thiol group on the activation peptide C5a (Fernandez & Hugli, 1977) was reported to be blocked by cysteine (Fernandez, 1978).

Component C5 loses all its haemolytic activity on conversion into C5b, and an open question remains as to why C5b is inactive.

In summary, component C5 has both similarities to, and differences from, component C3 with regard to mode of activation and functional expression. Continuing efforts need to be made to explore structural details of component C5 in order to gain an understanding of its role in the complement system.

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