The Biochemistry of Complement

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Complement is not a topic that features in many biochemical curricula, nor has it been investigated by Cambridge biochemists, so some justification is perhaps necessary for this choice of subject for the Gowland Hopkins Lecture.

This can be done from two points of view. Firstly, for the biochemist, complement is a highly complex group of serum proteins whose interactions lead through a series of steps to an attack mechanism on cell membranes. Some steps involve the activation of proteolytic zymogens, and included in the system are a correspondingly elaborate series of controls which cause inhibition or inactivation of the different components. Secondly, for the biologist, complement is an integral part of an animal's capacity to recognize foreign substances and to defend itself against infection by pathogenic organisms. The interest lies in trying to understand the latter in terms of the structure and biological activities of the many different proteins of the complement system.

The discovery of complement goes back 80 years, when Bordet (1896, 1898) working in Pasteur's laboratory in Paris first confirmed others' observations that serum from an animal, immunized with bacteria (he used Cholera vibrio) would, if added to the bacteria in vitro, kill them by agglutination and lysis. Then he found that if the serum was left at room temperature for a week or was heated briefly to 50–60°C, it would agglutinate the bacteria but not cause lysis and death. If added to a broth the agglutinated bacteria could still grow and form a lethal culture. If, however, Bordet added fresh serum from a non-immune animal to the agglutinated bacteria they were lysed. He postulated that two substances must be involved in this reaction, first the antibody [discovered 7 years earlier by Behring (Behring, 1890; Behring & Kitisato, 1890)], which is specific and causes agglutination, and secondly the non-specific substance, which is activated secondarily and causes lysis. The non-specific component he called alexin, but after further investigation (notably by Erhlich & Morgenroth, 1899a,b, 1900a,b) it became known as complement. It was soon found to be a mixture of substances, but, although it remained a valuable reagent for the pathologist and bacteriologist, it was not until the development of techniques of protein fractionation and structural analysis after the second war that complement became accessible to study by the biochemist and the full complexity of the phenomena became apparent. I hope to persuade you that complement is now a rich field of investigation for the protein chemist and enzymologist, for those interested in cell membrane structure and in biochemical genetics and no doubt for many others.
The starting point is Bordet’s original observation which showed that if heated immune serum is added to cells, say erythrocytes, they will be agglutinated. They can be washed free of serum protein and if fresh serum is then added the cells will be lysed, and the rate of lysis is in proportion to the amount of serum added. This is caused by the complement proteins in the blood and is used as an assay for whole complement or, in appropriate conditions, for any of the complement components. Some 20 proteins are now known to be involved. There is a sequential series of steps in the activation, which culminate in the rupture of the cell membrane. There are two pathways of initiating activation: one is the so-called classical pathway, which is started only by antibody after it has been aggregated by reaction with antigen (reviewed by Reid & Porter, 1975; Porter, 1977), and the second is the alternative pathway, which is started by other substances, such as polysaccharides, as well as by aggregated antibody (reviewed by Götte & Müller-Eberhard, 1976). Both pathways lead to the activation of the third component of complement, C3, and then follow a common course until cell lysis is caused by a complex of the last five components C5, C6, C7, C8 and C9. The mechanism of lysis is still uncertain, but it does not appear to be enzymic but is more probably an insertion of the complex into the membrane leading to its rupture (Mayer, 1972; Kolb & Müller-Eberhard, 1975).

The most obvious function of complement is that of an effector mechanism which, in an immune animal, leads to the destruction of invading organisms after their interaction with antibody. In addition to the lytic mechanisms, activation of complement produces peptides, which are chemotactic for phagocytic cells, and also altered proteins, which attach to the cell surface and promote adherence to and uptake by phagocytic cells, opsonization. The whole system constitutes an effective series of reactions for the destruction and elimination of bacteria and viruses.

The capacity of activated complement proteins, notably C3, to bind to cell surfaces and to promote interaction between cells (reviewed by Bianco & Nussenzweig, 1977) appears likely also to play a direct role in the immune response, in facilitating the interaction of the different classes of lymphocytes which is an essential part of the mechanism of antibody formation.

Most recently it has been shown that three complement proteins, C2, C4 and factor B, and possibly a fourth, C8, are coded for by structural genes that are part of the major histocompatibility complex on chromosome 17 in the mouse and chromosome 6 in man (reviewed by Hobart & Lachmann, 1976). This is a complex containing genes coding for the highly polymorphic histocompatibility antigens, which are present on the surface of all nucleated cells and which are responsible for the antigenic differences between cells from different individuals and hence for the rejection of tissue grafts within the same species. Also in this same complex are the genes (Ir genes) controlling immune responses to difference antigens and the genes coding for a set of cell surface glycoproteins, the Ia antigens, which are found principally on B lymphocytes and which are also believed to have a role in the immune response (Klein, 1975; Bodmer, 1977). The significance of the presence of structural genes coding for complement protein in this remarkable complex, which is only 1/3000th part of the human genome, is not clear. It has been suggested that many of these genes may have arisen by duplication and that all code for proteins with an important function in cell interaction through their presence in the cell surface.

It is probable that, although there is much uncertainty about the multiple roles of the complement proteins, they certainly have an essential part in an animal’s defence against infection, and this is confirmed by the rare cases of inherited deficiencies of different components. In component-C3-deficient patients, for example, repeated bacterial infection occurs as it does in agammaglobulinaemic patients who are unable to synthesize antibodies (Alper et al., 1976).

The complement system is highly complex and I propose to illustrate the biochemical approach to the structures and activation mechanism of the different components by concentrating on recent work on the initiation of the activation of the first component, C1, by antibody–antigen aggregates. Five proteins in addition to antibody are concerned, and the steps involved are shown in Fig. 1. When aggregated antibody is added to serum, the first component of complement, C1, binds and becomes an active proteinase. This
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Ab–Ag aggregates

\[ \text{C1}(q + r + s) \rightarrow \text{C1}(q + r + s) \]

\[ \text{C}2 \rightarrow \text{C}4 \rightarrow \text{C}4^2 \rightarrow \text{C}3 \]

Fig. 1. Activation of the early components of complement

The first component of complement (C1) binds to antibody–antigen (Ab–Ag) aggregates or antibody bound to cells and is activated through its subcomponents C1q, C1r and C1s. Activated subcomponent C1s is a proteolytic enzyme which hydrolyses components C2 and C4 which combine to give a complex enzyme C42. This is also a proteinase and converts component C3 into an activated form.

catalyses the conversion of the second and fourth components, C2 and C4, into forms that interact and together form another proteinase which converts component C3 into a reactive form. In the alternative pathway different steps lead to activation of component C3 and then subsequent reactions common to both pathways lead to activation of the later components and lysis of the cell if the antigen is a part of a cell surface. The cell surface itself does not appear to play an essential role, because activation of all the components occurs when they interact with antibody–antigen aggregates that have been formed from a pure protein antigen, such as ovalbumin. In both aggregates and antibody–coated cells an assembly of the components into macromolecular complexes appears to be an essential feature of the activation system.

The First Component, C1

Component C1 contains three proteins, C1q, C1r and C1s (Lepow et al., 1963), and in serum that contains 5mM-Ca²⁺, subcomponents C1r and C1s are in a tight complex containing two molecules of each giving a mol.wt. of about 350000 and possibly also an octomeric complex of double this size (Nagasaki et al., 1974; Sim, 1976). Interaction of this complex with subcomponent C1q (mol.wt. about 400000) is weak, and if a euglobulin precipitate of serum, which contains 90% of the component-C1 haemolytic activity, is fractionated on a column of Sepharose 6B in buffers at pH 5.5, the activity is eluted in the position of molecules of 400000 mol.wt., suggesting that under these conditions subcomponent C1q and the C1r–C1s complex are eluted together but show little or no interaction (Gigli et al., 1976). If undiluted serum is fractionated on a sucrose gradient, however, component-C1 haemolytic activity has a sedimentation coefficient of 19S (Naff & Ratnoff, 1968; Pickering et al., 1970; Lepow et al., 1963; Loos et al., 1976) (but lower in diluted serum), rather than 12S found for subcomponent C1q, C1r and C1s mixtures in 5M-Ca²⁺ (Lepow et al., 1963). It is possible therefore that in whole serum a fourth protein is associated with subcomponents C1q, C1r and C1s or perhaps that subcomponent C1q is present in a precursor form (Reid & Solomon, 1977), which interacts more strongly with the C1r–C1s complex and which is so labile as to be split on dilution or by other mild procedures. The weak interaction between subcomponent C1q and the C1r–Ca²⁺–C1s complex prevents the isolation of the unactivated C1
Table 1. Relative haemolytic activity of component C1 in a euglobulin precipitate of serum and in a mixture of purified subcomponents C1q, C1r and C1s

Mixtures of subcomponents C1q, C1r and C1s, when mixed in the same ratio as found in the serum euglobulin precipitate, have the same specific activity. Content is expressed in arbitrary units.

<table>
<thead>
<tr>
<th>Content of:</th>
<th>C1q</th>
<th>C1r</th>
<th>C1s</th>
<th>Haemolytic activity (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euglobulin precipitate</td>
<td>650</td>
<td>210</td>
<td>280</td>
<td>700,000</td>
</tr>
<tr>
<td>C1q, C1r, C1s mixture</td>
<td>100</td>
<td>36</td>
<td>44</td>
<td>107,000</td>
</tr>
<tr>
<td>Ratio euglobulin mixture</td>
<td>6.5</td>
<td>5.6</td>
<td>6.4</td>
<td>6.5</td>
</tr>
</tbody>
</table>

complex as such from serum, though the fraction from a Sepharose column containing component-C1 haemolytic activity contains only one obvious impurity. However, if this pool is fractionated on DEAE-cellulose columns after addition of EDTA (Lepow et al., 1963), subcomponents C1q, C1r and C1s can be obtained as single components free of detectable impurities (Gigli et al., 1976). Serum contains more than 1 g of proteolytic inhibitors/100 ml (Helmburger, 1974), all of which are left in the supernatant when the initial euglobulin fraction is precipitated at pH 7.4. Unless other inhibitors, such as di-isopropyl phosphorofluoridate, are added to the serum initially and are added again at each stage of the fractionation, spontaneous activation of component C1 occurs during the isolation. Traces of proteinases or their zymogens apparently persist throughout. With these precautions, unactivated subcomponents C1q, C1r and C1s can be isolated and can be remixed in the proportions found in the euglobulin precipitate (i.e. 1 mol of C1q to 2 mol each of C1r and C1s). When the haemolytic activity of such a mixture is compared with that of the crude precipitate, it is found to be the same within the accuracy of the assays (Gigli et al., 1976) (Table 1). As 90% of the component C1 haemolytic activity of the original serum is found in the euglobulin precipitate, this is good evidence that the whole of this activity in the serum is accounted for by the three subcomponents and that it is unlikely that a fourth component is involved.

If no precautions are taken to prevent proteolysis or if component C1 is eluted from antibody-antigen aggregates, it is in the activated form C1T. Both subcomponent C1Tq and C1Ts have become active proteinases, but no difference can be detected in subcomponent C1q which has no recognizable catalytic activity.

Interaction of component C1 with antibody

There is only very weak interaction between subcomponent C1q and monomer IgG,* but strong binding after the IgG has been aggregated (Müller-Eberhard & Kunkel, 1961). The C1r-C1s complex does not bind to IgG, aggregated or not, and hence the binding of component C1 to aggregated antibody is believed to be through subcomponent C1q. The binding site on the IgG is in the second constant domain Cγ2, the N-terminal half of the Fc fragment, as the Fab fragment, which has lost the Cγ3 domain, retains the capacity to activate complement after aggregation with antigen (Connell & Porter, 1971; Colomb & Porter, 1975), but the smaller (Fab')2 fragment, which has lost the whole of the Fc fragment including both the Cγ2 and Cγ3 domains, will no longer activate component C1 after aggregation (Reid, 1971). In agreement with this, heat-aggregated fragment Fc, but not fragment (Fab')2, will activate component C1 (Taranta & Franklin, 1961; Ishizaka et al., 1962). The Cγ2 domain contains the polysaccharide attached by a glycosylamine band to an asparagine residue (Press, 1965), and it has been reported that removal of all the carbohydrate, except the N-acetylglucosamine residue bound to the asparagine, by a series of glycosidases lowers the complement-fixing ability of rabbit anti-(sheep erythrocyte) antibodies by 80% (Koide et al., 1977). If the

* Abbreviation: IgG, immunoglobulin G.
polysaccharide itself was an essential part of the binding site for subcomponent Clq, complete loss of complement activation would be expected, and these results suggest that the binding site is more likely to be to a portion of the peptide chain closely associated with the polysaccharide. The crystallographic structure reported by Huber and colleagues (Diesenhofer et al., 1976) shows the polysaccharide clearly defined and bound, probably by hydrophobic interaction, to one side of the C1q2 domain. Removal of the polysaccharide could well influence the configuration of this section of peptide chain, particularly if, in the aggregation of IgG necessary for binding of subcomponent Clq, a conformational change occurs in this section.

Structure of subcomponents Clq, Clr and C1s

Subcomponent Clq. This is a large protein of mol.wt. 400000 (Calcott & Müller-Eberhard, 1972; Reid et al., 1972), and it was apparent that it was an unusual protein when analysis showed that it contained hydroxylysine and hydroxyproline (Müller-Eberhard, 1968), amino acids that are usually found only in collagen and basal membrane proteins. In a dissociating medium such as 6M-guanidine, two subunits can be recognized, both with mol.wt. about 48000, and, on reduction, three peptide chains, A, B and C are obtained, again of similar size, 23000–25000 mol.wt. In spite of the similar sizes, both the subunits and the peptide chains can be separated easily on electrophoresis in polyacrylamide gels in buffers containing sodium dodecyl sulphate, probably because of differences in the carbohydrate content. It was reported that the apparently larger subunit, as judged by rate of movement on polyacrylamide-gel electrophoresis, contained the A-B subunit to the C-C subunit (Yonemasu & Stroud, 1972) and we could barely detect the C-C subunit (Reid et al., 1972). The explanation lay in the aggregation of the C-C subunit if the sample was loaded on to the electrophoresis


B-Chain  Glu-Leu-Ser-Cys-Thr-Gly-Pro-Hyp-(Gly-X-Y)$_{24}$-Gly-Asp-Tyr-Lys

C-Chain  Asn-Thr-Gly-Cys-Tyr-(Gly-X-Y)$_{24}$-Gly-Arg-Tyr-Lys-Gln-Lys-

C-Chain  Asn-Thr-Gly-Cys-Tyr-(Gly-X-Y)$_{24}$-Gly-Arg-Tyr-Lys-Gln-Lys-

Fig. 2. N-Terminal sequences and interchain disulphide bonds of Clq peptide chains

The only interchain disulphide bonds between the A–B and C–C peptide chain of subcomponent Clq are four residues from the N-termini of the chains. The Gly-X-Y collagen-like sequence begins at residue 9 in the A-chain, residue 6 in the B-chain and residue 3 in the C-chain (Reid, 1976; K. B. M. Reid, unpublished work).
gels containing salt (Plate 1). In spite of the urea and sodium dodecyl sulphate present, the C–C subunit aggregated and barely entered the gel if salt was present. In the absence of salt a 2:1 ratio of the A–B subunit to the C–C subunit was found. The peptide chains did not show this effect and equimolar ratios of the A-, B- and C-chains were present (Reid et al., 1972). When the correct molecular weights were also obtained, by rate of elution from Sephadex columns equilibrated with solutions of 6M-guanidine, it became clear that the Clq molecule contained six each of the A-, B- and C-chains, which were joined by disulphide bonds to give six A–B and three C–C subunits (Reid & Porter, 1976).

Amino acid-sequence analysis has shown that each of the A-, B- and C-chains has a typical collagen-like sequence of 78 residues, starting close to the N-terminus. Glycine occurs in every third position and all the hydroxyamino acid residues are present in this section (Reid, 1974, 1976). Many of the hydroxylysine residues have a glucosyl-galactosyl disaccharide attached (Yonemasu & Stroud, 1972; Calcott & Müller-Eberhard, 1972) again as in collagen. Though the sequences in the three peptide chains are very similar, they are not identical. There is one interchain disulphide bond in each subunit and this is at the N-terminus of the chain (Reid, 1976) (Fig. 2).

The collagen molecule is formed from three chains associated in a major helix and joined by both covalent and non-covalent bonds (reviewed by Traub & Piez, 1971). Evidence that such a triple-chain structure was present in subcomponent Clq came from the susceptibility to digestion by the specific enzyme collagenase and more definitely from the presence in the circular-dichroism spectrum of a peak at 215–230 nm, which is lost on warming to 50°C. This is characteristic of the native collagen triple-chain helical structure and makes it most probable that a similar structure is present in subcomponent Clq (Brodsky-Doyle et al., 1976). Fig. 3 shows that this peak is visible in the whole molecule and is more evident in a peptic digest that destroys the non-collagenous C-terminal half of the peptide chains and leaves the N-terminal halves containing the collagen-like sequences intact. Collagenase digestion gives a complementary fragment in which the sections containing the non-collagenous-like sequences are undigested and remain as globular fragments of low solubility (Reid et al., 1972).

Electron micrographs of subcomponent Clq show an unusual structure resembling a bunch of six tulips held together by their stems (Svehag & Bloth, 1970; Shelton et al., 1972; Knobel et al., 1975), suggesting a six-unit structure, each unit presumably formed from three peptide chains as the collagen model suggests (Plate 2). Reconciliation of the nine subunits with the six units can be made if there are three pairs of triple chains as shown in Fig. 4. Non-covalent linkage of these three pairs will give the whole molecule (Reid & Porter, 1976) as shown in Fig. 5. Measurement of the stems from the electron micrographs and comparison with the sizes calculated from the known dimensions of the amino acid residues in the collagen triple helix show close agreement.

One uncertainty was the bend on the stems about half way along their length, as this has not been seen in collagen fibrils. However, when the sequence of the collagenous section of the C-chain was completed, an alanine residue replacement for a glycine residue in position 36 was found in contrast with the A- and B-chains (Reid, 1977). Molecular models showed that such a replacement would cause a bend in the fibril similar to that seen in the pictures. The good agreement between the chemical and physical parameters and the electron-microscopic appearance of subcomponent Clq make it probable that the structure shown in Fig. 5 is substantially correct.

Further similarity between subcomponent Clq and collagen has come from the biopsynthetic studies of component C1 by fibroblast cultures (Reid & Solomon, 1977). It was found that component-C1 haemolytic activity increased linearly with time in the supernatant fluid of such cultures. Radioactive amino acids were incorporated into subcomponents Clq, Clr and Cls, as judged by the radioactivity found in the specific precipitates formed with antisera to each of these three proteins. When these specific precipitates were run on polyacrylamide gels in buffer containing sodium dodecyl sulphate, the radioactivity peaks in the precipitate of subcomponents Clr and Cls coincided with the position of the carrier Clr or Cls protein, whether reduced or unreduced.

1977
EXPLANATION OF PLATE I

Electrophoresis of samples of subcomponent Clq in increasing salt concentrations on polyacrylamide gels in sodium dodecyl sulphate-containing buffers

Electrophoresis on 5.6% polyacrylamide gels, run in buffer containing 0.2% sodium dodecyl sulphate of subcomponent Clq with increasing ionic strengths of the applied sample. This shows the decreasing content of the Clq subcomponent of apparent mol. wt. 54000 with increasing salt concentration, from left to right, of the sample loaded. This subcomponent aggregates at higher salt concentrations in spite of the urea and sodium dodecyl sulphate present. [This is taken from Reid & Porter (1976).]
Electron micrograph of subcomponent C\1q

The bar represents 20 nm. [This is taken from Knobel et al. (1975).]
Fig. 3. Circular-dichroism spectra of (a) native subcomponent C1q and after heating to 52°C and 65°C and (b) the peptic-digest fragment of subcomponent C1q (native and after heating to 65°C).

This absorption maximum at 220–230 nm is typical of collagen and, as in collagen, it is lost on heating. The peak is more marked in the peptic fragment which contains only the collagen-like tail structure of subcomponent C1q. The data are taken from Brodsky-Doyle et al. (1976).

However, for subcomponent C1q, the radioactive proteins were significantly slower-moving and therefore had apparent molecular weights higher than the corresponding subunits and peptide chains of the carrier protein. This suggests that subcomponent C1q may, like collagen, be secreted as a higher-molecular-weight precursor form, which is split either at the cell surface or possibly in the plasma or even after interaction with aggregated antibody. If either of the last two possibilities are correct, the peptide bond broken would have to be very labile, as the only suggestion of a higher-molecular-
Equimolar amounts of A-, B- and C-chains
(each 22000-24000 mol.wt.)

Reduction or Oxidation

A
B
C

Collagen-like region of 78 residues
Non-collagen-like region of 103-108 residues

2-8 residues

Collagen-like region
Non-collagen-like region

Globular
C-terminal
regions

Helical ends of structural units
form fibril to yield intact molecule
of 410 000 mol.wt.

Fig. 4. Proposed chain, subunit and dimer unit structure of subcomponent C1q

Each chain of the C–C dimer associates non-covalently with an A–B dimer to give a
dimer unit containing two A-, two B- and two C-chains. [Taken from Reid & Porter
(1976).]

weight form of subcomponent C1q in plasma is the sedimentation value of the whole
component C1, which, as mentioned above, has been reported to be 19S in whole serum
but falls to 14S or 16S on dilution. Proof, by isolation, of the existence of such a
precursor C1q molecule would necessitate exceptional precautions to prevent the
proposed proteolysis.

Subcomponents C1r and C1s. These proteins are very similar and have much simpler
structures than subcomponent C1q, namely single peptide chain structures of mol.wt.
83000 and differing in that subcomponent C1r behaves as a dimer and subcomponent
Region of the molecule thought to be left intact after collagenase digestion, i.e. the globular peripheral portions

Region of molecule thought to be left intact after pepsin digestion at pH 4.4, i.e. the six connecting strands plus fibril-like end piece

Fig. 5. Proposed model of human subcomponent C1q

---, Portions of molecule pointing towards reader; ----, portions of molecule pointing inwards away from reader. Dimensions are averages of those published by Shelton et al. (1972), as estimated from electron-microscopy studies. ~, Proposed triple-helix sections, i.e. collagen-like regions of the molecule. Length of collagen-like fibre + fibril-like end piece = 11.5 nm + 11.2 nm = 22.7 nm (115 Å + 112 Å = 227 Å) and the length of the triple helix proposed from sequence studies = 8 nm x 0.29 mm = 23.2 nm (80 Å x 2.9 Å = 232 Å). [Taken from Reid & Porter, (1976).]

C1r as a monomer in solution, in the presence or absence of Ca²⁺ (Sim et al., 1977; Valet & Cooper, 1974a,b; Ziccardi & Cooper, 1976). When in solution together with Ca²⁺, subcomponents C1r and C1s form a tetrameric complex of about 350000 mol.wt. After activation they remain as a complex, but, each peptide chain has been split into two chains, 'a' (56000 mol.wt.) and 'b' (27000 mol.wt.) joined by disulphide bonds. Both are now active proteinases, the 'b'-chains containing the catalytic site. This is easily identified, as both are inactivated by di-isopropyl phosphorofluoridate (Naff & Ratnoff, 1968; Sakai & Stroud, 1974; Takahashi et al., 1975) and if a radioactive reagent is used, it can be shown that 1 mol of di-isopropyl phosphorofluoridate reacts with 1 mol of the 'b'-chains (Gigli et al., 1976; Sim & Porter, 1976). Subcomponents C1r and C1s behave therefore as serine esterase-type proteinases and their classification is supported by the N-terminal sequence of the 'b'-chains in which the first 20 amino acids are very similar to each other and show an obvious homology with the equivalent sequences of other serine esterase-type proteinases (Sim et al., 1977) (Fig. 6).

The structural similarities of subcomponents C1r and C1s are so great as to suggest that they have arisen by gene duplication, but when their enzymic specificities are examined, there are striking differences. Subcomponent C1s can be isolated with suitable precautions as a stable molecule (Gigli et al., 1976; Arlaud et al., 1977a,b) and in solution it is activated by subcomponent C1r but not by subcomponent C1r. Extrapolation from reactions in solution to these where the subcomponents are bound to insoluble antibody aggregates is uncertain, but it is probable that also in the bound state subcomponent C1s is not autoactivatable, but is activated by subcomponent C1r, as inactivation of subcomponent C1r before adsorption on the complex prevents activation of subcomponent C1s. In solution subcomponent C1s will hydrolyse components C4 and C2 but not subcomponent C1r. It will also hydrolyse amino acid ester substrates, showing a plasmin-like specificity most effective on lysine esters (Sim et al., 1977).
Residue no. & (5) & (10) & (15) & (20)

Human C\(\text{I}\)r 'b'-chain & Ile-Ile-Gly-Gly-Gln-Lys-Ala-Lys-Met-Gly-Asn-Phe-Pro-Trp-Gln-Val-Phe-Thr-Asn-Glx & 

Human C\(\text{I}\)s 'b'-chain & Ile-Ile-Gly-Ser-Asp-Ala-Asp-Ile-Lys-Asn-Phe-Pro-Trp-Gln-Val-Phe-Phe-Asp-Asn & 

Bovine chymotrypsin A & Ile-Val-Asn-Gly-Glu-Glu-Ala-Val-Pro-Gly-Ser-Trp-Pro-Trp-Gln-Val-Ser-Leu-Gln-Asp & 

Bovine trypsin & Ile-Val-Gly-Gly-Tyr-Thr-Cys-Gly-Ala-Asn-Thr-Val-Pro-Tyr-Gln-Val-Ser-Leu-Asn-Ser & 

Bovine thrombin B-chain & Ile-Val-Glu-Gly-Gln-Asp-Ala-Glu-Val-Gly-Leu-Ser-Pro-Trp-Gln-Val-Met-Leu-Phe-Arg & 

Human plasmin B-chain & Val-Val-Gly-Gly-Cys-Val-Ala-His-Pro-His-Ser-Trp-Pro-Trp-Gln-Val-Val-Leu-Leu-Arg &

Fig. 6. Comparison of the N-terminal sequences of subcomponents C\(\text{I}\)r and C\(\text{I}\)s 'b'-chains with the corresponding polypeptide chains of other serine proteinases

Residues that are identical in both subcomponents C\(\text{I}\)r and C\(\text{I}\)s 'b'-chain are shown underlined. Those identical with either subcomponent C\(\text{I}\)r or C\(\text{I}\)s 'b'-chain are shown in italic typeface. [Taken from Sim et al. (1977).]
In contrast, subcomponent Cir will not catalyse the hydrolysis at a significant rate of any protein or peptide substrate tested so far, except subcomponent Cls (Sim et al., 1977). It will not hydrolyse subcomponent Clr and hence is not autoactivatable in solution. However, preparations of subcomponent Clr stable over many hours at neutral pH and 37°C have not been obtained. Varying rates of activation are found and bear no relation to the haemolytic activity. As addition of equal weights of subcomponent Cir to the Clr subcomponent does not increase activation rates, it is probable that the apparent autoactivation observed is due to traces of other proteinases or activatable zymogens still present. Antibody–antigen complexes bind subcomponent Clr if subcomponent Clq is present, but activation of subcomponent Clr does not occur at a higher rate than in free solution. If, however, subcomponent Cls and Ca²⁺ are also present, activation of both subcomponent Clr and Cls occurs rapidly. Activation of subcomponent Clr occurs at the same rate when subcomponent Cls is replaced by Cls or by Clr that has been fully inactivated by addition of di-isopropyl phosphorofluoridate. Hence in the activation complex, subcomponent Cls is essential, but has no catalytic function. If 10⁻⁶ di-isopropyl phosphorofluoridate is present during the activation, the activation of subcomponent Cls by Cir is almost completely inhibited, but subcomponent Clr becomes fully activated (A. W. Dodds, R. B. Sim & R. R. Porter, unpublished work). This again suggests that subcomponent Cir does not activate subcomponent Clr. It is possible that the binding of subcomponent Cir with Cls and Ca²⁺ to subcomponent Clq bound to the antibody aggregates leads to the exposure of the catalytic site on subcomponent Clr, which splits other Clr molecules giving subcomponent Cir which then splits subcomponent Cls. More direct evidence on this complex interaction is being sought by using site-directed inhibitors.

Component C4

The next step in the complement-activation system is the hydrolysis of components C4 and C2 by bound subcomponent Cls to give products that interact and form the C42 complex, which is also an active protease. Component C4 has a three-chain structure (Schreiber & Müller-Eberhard, 1974; Nagasawa & Stroud, 1976), the approximate molecular weights being for the α-chain 90000, for the β-chain 70000 and for the γ-chain 30000, and subcomponent Cls releases a 6000-mol.wt. peptide from the N-terminus of the α-chain (Schreiber & Müller-Eberhard, 1974; Bolotin et al., 1977) leaving component C4 (or C4b). The peptide chains of component C4 are held together firmly by strong non-covalent bonds as well as by disulphide bonds, and separation of the three peptide chains after reduction requires strongly dissociating conditions, such as 6M-guanidine or 1% sodium dodecyl sulphate. Under the latter conditions the three chains were separated on hydroxyapatite columns (Gigli et al., 1977) and the N-terminal sequences determined. Similar results have been obtained under different conditions by Bolotin et al. (1977). The N-terminal 20 residues of the γ-chain (Gigli et al., 1977) show no relation to the sequence of the Ia antigens (Springer et al., 1977) and hence it is improbable that these two peptide chains have arisen by gene duplication, though they are of similar size and both Ia antigens and at least one peptide chain of component C4 are coded by genes that are close to each other in the major histocompatibility gene complex.

Component C2

Component C2 has proved the most difficult of the early components of complement to isolate, as it is present in much lower amounts in serum (20–30mg/litre) and is perhaps the most easily hydrolysed. It shows marked similarity in size and structure to a component of the alternative pathway, Factor B, which is present in 10–20-fold higher concentration and is difficult to separate from component C2. A recent abstract reported the isolation of component C2 by affinity chromatography (Nagasawa & Stroud, 1977). It has also been purified by a combination of ion-exchange and exclusion chromato-
graphy, including one step intended to be affinity chromatography but which on further investigation seemed more likely to be an unusual type of ion-exchange chromatography (M. A. Kerr & R. R. Porter, unpublished work). Component C2 has mol. wt. about 100000 (Polley & Müller-Eberhard, 1968; Nagasawa & Stroud, 1977; M. A. Kerr & R. R. Porter, unpublished work), and, like all the other components studied, is a glycoprotein. It has only one polypeptide chain and is split by subcomponent C1s to give a fragment, which associates with component C4 and is of about 70000 mol. wt., and an inactive peptide of 30000 mol. wt. (Polley & Müller-Eberhard, 1968). A recent paper reports slow reaction of both the split and unsplit component C2 with di-isopropyl phosphorofluoridate and suggests that component C2, also, is a serine esterase-type proteinase (Medicus et al., 1976). Component C2 has several unusual features, such as the 10–20-fold increase in haemolytic activity of human, but not of guinea-pig, component C2 on treatment with weak iodine solution (Polley & Müller-Eberhard, 1967). The availability of an adequate method of preparation should now make a detailed investigation possible. In particular its specific interaction with component C4 in the activation pathway is most unusual and an understanding of this interaction should help to clarify the structural basis of the catalytic activity necessary for the activation of component C3.

Assembly of the Initial Complement Complex

As has been described, subcomponent C1q binds to aggregated antibody through the second constant domain. There is evidence that, if subcomponent C1q is treated with collagenase destroying the stems of the molecule but leaving the globular heads intact, the heads will still bind to aggregated antibody (Knobel et al., 1975). Binding of subcomponent C1q to aggregated antibody is likely therefore to be through the heads. A peptic digest of subcomponent C1q leaves the stems intact, but destroys the heads and such fragments will inhibit the haemolytic activity of the Clr$_2$–Ca$^{2+}$–Cis$_2$ tetramer if added to antibody-coated erythrocytes with bound subcomponent C1q before addition of subcomponents Clr and Cis (Reid et al., 1977). Hence it is probable that subcomponents Clr and Cis bind to subcomponent C1q through the collagenous tails. It is essential that the peptic digest is added first, as, when subcomponents Clr and Cis are added first, their haemolytic activity cannot be inhibited by addition of the peptic digest. This suggests that Clr–Ca$^{2+}$–Cis bind firmly possibly because of a conformational change that takes place in the collagen stems of subcomponent C1q after its binding to antibody. The activation of subcomponents Clr and Cis, which follows their attachment, leads to even firmer interaction. Subcomponent Clr, but not subcomponent Cis, will bind to the subcomponent C1q–antibody complex and hence the tetrameric Clr$_2$–Ca$^{2+}$–Cis$_2$ complex is likely to bind to subcomponent C1q through subcomponent Clr. Optimal component C1 haemolytic activity is obtained when the molar ratios of subcomponents C1q/Clr/Cis is 1:4:4 (Gigli et al., 1976) (Fig. 7), suggesting that two tetrameric Clr$_2$–Ca$^{2+}$–Cis$_2$ complexes may bind to each C1q molecule attached to aggregated antibody.

The role of the cell membrane in the complement-activation sequence is uncertain, as complete activation of complement will result whether an antibody–protein antigen precipitate or antibody-coated erythrocytes are added to fresh serum. Quantitative data are only just becoming available (J. G. Goers & R. R. Porter, unpublished work) and they suggest that per 1000 antibody molecules, cell-bound antibody is about twice as effective as antibody aggregates in binding subcomponent C1q. This result could well be due to differences in the availability of the antibody C$_{1q2}$ domain, which may be more easily approached by the heads of the C1q molecule when the antibody is spread on a surface than when aggregated in a precipitate. Binding of component C4 in the presence of maximum amounts of component C1 bound to antibody is 20–30 times higher when the antibody is on an erythrocyte rather than in aggregates. However, the haemolytic titre and therefore efficiency, in the activation scheme, of C4 molecules is directly proportional to the bound C1 molecules and not to the total bound C4 molecules present. This suggests that much component C4 may be adsorbed on to the erythrocyte surface, but only that bound directly to the component C1–antibody complex or perhaps
at the cell surface close to the complex is effective in causing haemolysis (see also OpferKuch et al., 1971).

There is an early report that if an antibody–antigen precipitate is added to fresh serum and the precipitate then centrifuged down and washed, and subsequently split into Fab

![Graph](image1.png)

**Fig. 7.** Assay of component-C1 haemolytic activities on addition of increasing amounts of subcomponent C1r plus C1s to subcomponent C1q and the effect of different ratios of C1r/C1s

Δ, Subcomponent C1q with addition of a mixture of subcomponents C1r/C1s in molar ratio 1:1; ○, subcomponent C1q with addition of mixtures of C1r/C1s in molar ratio 2:1; ●, subcomponent C1q with addition of mixture C1r/C1s in molar ratio 1:2; □, subcomponent C1q with addition of C1s alone. The abscissa gives the molar ratio of C1r or C1s, whichever is in the least concentration, to C1q. (Taken from Gigli et al., 1976.)

![Diagram](image2.png)

**Fig. 8.** Suggested assembly of early components of complement on to antibody molecules bound to a cell-surface antigen

The heads of C1q molecules bind to the C_{1q2} domain of 2 or more antibody molecules and at least one tetramer C1r_{2}-Ca^{2+}-C1s_{2} binds to the collagen-like tail of subcomponent C1q. This leads to conversion of subcomponent C1s into a proteolytic enzyme which hydrolyses components C4 and C2, and together they form another proteinase C42. Early qualitative evidence (Chan & Cebra, 1968) suggests that this may bind to the C_{1q1} domain of the antibody molecules, but proof of this is not yet available.
and Fc fragments, component C4 can be detected on the Fab fragment and not on the Fc fragment (Chan & Cebra, 1968). If quantitative experiments confirm these results, effective C4 molecules may be those that bind to the Fab fragment, presumably through the C4I domain, and then interact with component C2. Localization of these components to give high enzyme concentration at the antibody surface may be essential for the continuation of the activation process as, in vivo, activation is occurring in suspensions in blood or at tissue surfaces bathed in blood or lymph which contain high concentrations of proteolytic inhibitors. Only in the immediate environment of the antibody will the proteolytic activities exceed the inhibitory capacity of the plasma. Much more evidence is required, but a possible model for the initial steps of the activation of complement by aggregated antibodies and involving six proteins is beginning to take shape and is shown diagrammatically in Fig. 8.

Conclusions

The activation of the early components of complement in the classical pathway by interaction with aggregated antibody is initiated by the binding of component C1 to the second constant domain of the antibody. It is the C1q subcomponent of component C1 that binds to the antibody, and it is still unclear as to whether a binding site for sub-component C1q is created or exposed by the aggregation or whether the bringing together of antibody molecules by aggregation makes possible multiple binding and hence higher affinity by the hexameric C1q molecule. Both features may occur in the interaction, which leads in turn to much stronger binding of one or two C1r2-Ca2+-C1s2 tetrameric complexes to the bound subcomponent C1q. The unique half-collagen-half-globular structure of subcomponent C1q leads to interaction of the globular heads of subcomponent C1q with the C4 region of the antibody and consequent interaction of C1r2-Ca2+-C1s2 with the collagen-like stems of subcomponent C1q through subcomponent C1r. This binding of the C1r2-Ca2+-C1s2 complex causes conversion of sub-component C1r into C1r, a proteinase that catalyses the activation of subcomponent C1s to another proteinase, subcomponent C1s. Both subcomponents C1r and C1s behave as typical serine esterase-type proteinases, and although they are very similar in structure, these two enzymes differ obviously in specificity. Subcomponent C1s hydrolysates both components C4 and C2, releasing a 6000-mol.wt. peptide from the -chain of component C4 and a 30000-mol.wt. peptide from the single-chain component C2 molecule. The activated forms of components C4 and C2 interact to form a complex which perhaps binds to the antibody C4I domain and catalyses the activation of component C3 and so on along the complement sequence. If the antigen to which the antibody is bound is a cell, lysis will finally occur.

The invitation to give this lecture stated that Hopkins Lecturers were encouraged 'to look ahead to future research or to the effect of current research on other disciplines'. I hope that I have made obvious the potential for future research for biochemists in the field of complement, where the work is only now beginning. The necessity for parallel investigation by the geneticists and immunologists is I think equally clear, but the biochemists can make an essential contribution to the full understanding of this complex phenomenon.

The bibliography shows that many of the experiments described in this lecture were carried out by colleagues in the M.R.C. Immunochemistry Unit in Oxford and I acknowledge particularly the major contribution of Dr. K. B. M. Reid.

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