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Structure and Activation of Complement Components C2 and Factor B

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Structure and activation of complement components C2 and Factor B

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The activation of complement is initiated by two independent pathways. Each leads to the formation of a complex protease, C3 convertase, with equivalent specificity and function but different composition. The convertase derived from the classical pathway is composed of complement components C4 and C2 while that from the alternative pathway consists of components C3 and Factor B. C2 and Factor B contain the catalytic site of each convertase respectively. The amino acid sequence of Factor B has been determined. Limited sequence of CNBr-peptides isolated from C2 has also been obtained. The two enzymes are shown to be homologous and to represent a novel type of serine proteinase, characterized by their unusual structure and mechanism of activation, when compared to known serine proteinases.

The complement system is an important effector mechanism of the organism's defence against infection. It consists of an enzyme cascade following two initial pathways leading to the activation of components C3 and C5. Component C3 is a protein central to the biological activities of complement. These activities are expressed after proteolytic cleavage of C3 by a complex enzyme C3 convertase, the nature of which depends on the pathway of activation. The product of this cleavage, $C\bar{3}$, associates with the same enzyme and enables it to cleave C5 leading to the activation and assembly of a macromolecular complex of the late components of complements (C5–C9) (for review see Reid & Porter 1981; Lachmann 1979).

In the classical pathway the C3 convertase is derived from complement components C4 and C2. After proteolytic activation by the C1 complex $C\bar{4}$ binds covalently to antibody–antigen aggregates or antibody coated cells, and $C\bar{2}$ associate non-covalently with $C\bar{4}$ to form the C3 convertase. In the alternative pathway activated C3 and Factor B form the C3 convertase. Similarly to $C\bar{4}$, $C\bar{3}$ can be covalently linked to polysaccharide, antibody aggregates or activating particles. Factor $\bar{B}$ is bound non-covalently to $C\bar{3}$. C2 and Factor B are homologous in function and structure and are responsible for the catalytic activity of the classical and the alternative pathway convertase respectively. Both are encoded by closely linked genes in the major histocompatibility locus.

There were conflicting reports on the effect of diisopropylphosphofluoridate, a general inhibitor at serine proteinases, on the activity of the convertases (Medicus *et al.* 1976; Vogt *et al.* 1977). Because the catalytic polypeptide chains of the convertases are about twice the length of the catalytic peptides of all other serine proteinase there were doubts as to the nature of these enzymes.

The study of the chemical structure of the proteolytic component forming convertases is essential to the understanding of their functional importance.

Factor B

Factor B in its zymogen form is a serum glycoprotein of about 90000 M_r. It interacts reversibly with complement component $C\bar{3}$ in the presence of Mg^{2+} and is activated by factor $\bar{D}$, a serine proteinase of the alternative pathway. Activation requires cleavage of a single peptide bond resulting in two non-covalently linked fragments: the N-terminal Ba fragment with a molecular mass of about 30000 Da and the C-terminal Bb fragment of approximately 60000 M_r. The latter fragment is responsible for catalytic activity and in association with $C\bar{3}$ forms the complex proteinases C3 convertase ($C\bar{3}\bar{B}$) and C5 convertase ($[C\bar{3}]_n\bar{B}$).

```
1           10          20          30          40
TPWSLARPQGSCSLEGVEIKGGSFRLLQEGQALEYVCPSG

41          50          60          70          80
FYPYPVQTRTCRSTGSWSTLKTQDQKTVRKAECRAIHCPR

81          90          100         110         120
PHDFENGEYWPRSPYYNVSDEISFHCYDGYTLRGSANRTC

121         130         140         150         160
QVNGRWSGQTAICDNGAGYCSNPGIPIGTRKVGSQYRLED

161         170         180         190         200
SVTYHCSRGLTLRGSQRRTCQEGGSWSGTEPSCQDSFMYD

201         210         220         230         240
TPQEVAEAFLSSLTETIEGVDAEDGHGPGEQQKRKIVLDP

241         250         260         270         280
SGSMNIYLVLDGSDSIGASNFTGAKKCLVNLIEKVASYGV

281         290         300         310         320
KPRYGLVTYATYPKIWVKVSEADSSNADWVTKQLNEINYE

321         330         340         350         360
DHKLKSGTNTKKALQAVYSMMSWPDDVPPEGWNRTRHVII

361         370         380         390         400
LMTDGLHNMGGDPITVIDEIRDLLYIGKDRKNPREDYLDV

401         410         420         430         440
YVFGVGPLVNQVNINALASKKDNEQHVFKVKDMENLEDVF

441         450         460         470         480
YQMIDESQSLSLCGMVWEHRKGTDYHKQPWQAKISVIRPS

481         490         500         510         520
KGHESCMGAVVSEYFVLTAAHCFTVDDKEHSIKVSVGGEK

521         530         540         550         560
RDLEIEVVLFHPNYNINGKKEAGIPEFYDYDVALIKLKNK

561         570         580         590         600
LKYGQTIRPICLPCTEGTTRALRLPPTTTCQQQKEELLPA

601         610         620         630         640
QDIKALFVSEEEKKLTRKEVYIKNGDKKGSCERDAQYAPG

641         650         660         670         680
YDKVKDISEVVTPRFLCTGGVSPYADPNTCRGDSGGPLIV

681         690         700         710         720
HKRSRFIQVGVISWGVVDVCKNQKRQKQVPAHARDFHINL

721         730
FQVLPWLKEKLQDEDLGFL
```

Figure 1. Amino acid sequence of factor B. The half cystine residue at position 267 carries the only free sulphydril group. Asparagine residues 97, 117, 260 and 353 have polysaccharide groups attached. Single letter code for amino acids: A, Ala; B, Asx; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; X, unknown; Y, Tyr; Z, Glx.

The complete amino acid sequence of Factor B has been determined and consists of 739 residues as shown in figure 1. Activation by factor $\bar{D}$ results in the cleavage of a single peptide bond between Arg-234 and Lys-235, and the production of fragment Ba consisting of 234 residues and the catalytic chain Bb composed of 505 amino acids.

The sequence of fragment Bb was first obtained by protein chemistry (Christie & Gagnon 1982, 1983; Gagnon & Christie 1983). This includes isolation and analysis of peptides

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

generated by cleavage at Met residues by CNBr, Arg residues by clostripain and trypsin, Glu residues by *Staphylococcus aureus* V-8 proteinase, between Asn-Gly by hydroxylamine and between Asp-Pro with dilute acid.

Short amino and carboxyl-terminal sequences of Ba were also obtained (Christie & Gagnon 1982) but the amino acid sequence of the segment covering residues 23–198 presented in figure 1 was derived from the nucleic acid sequence of a cDNA clone (Morley & Campbell 1984). Nucleic acid sequence of the gene coding for Factor B has also been determined (Campbell *et al.*, this symposium) and is in agreement with the sequence obtained by protein chemistry with the exception that the DNA sequence indicates the presence of an isoleucine residue at position 272 instead of a threonine residue. The complete sequence of Factor B has previously been reported briefly (Mole *et al.* 1983).

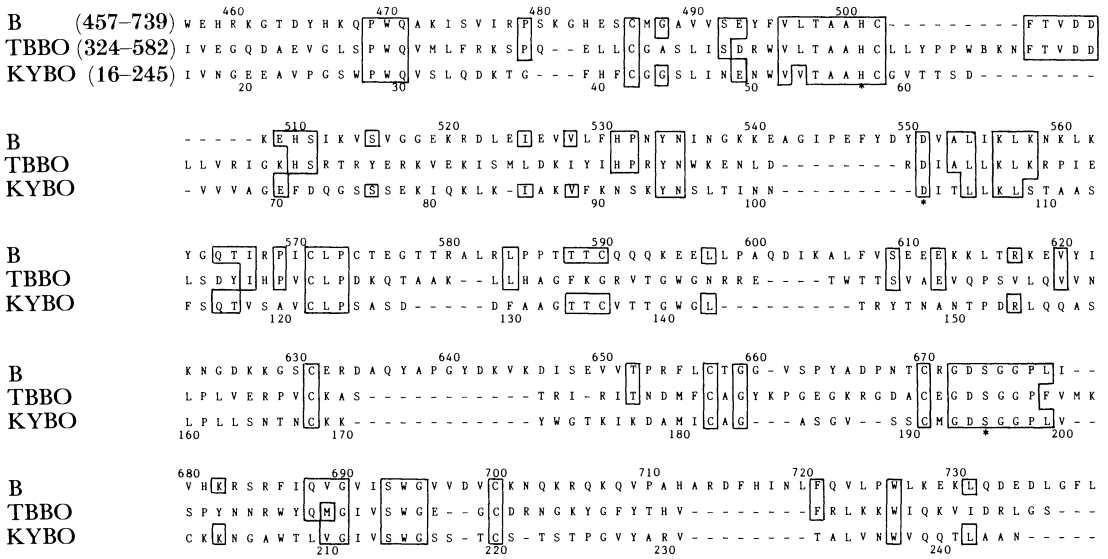

FIGURE 2. Alignment of the serine proteinase portion of Factor B with bovine thrombin and chymotrypsin. Sequence data and alignment for thrombin (TBBO) and chymotrypsin (KYBO) are from Young *et al.* (1978). The residue numbering indicated above is that of Factor B, the one below is that of chymotrypsinogen. Residues identical to Factor B are boxed. Asterisk denotes the position of active site residues.

Both fragments Ba and Bb carry carbohydrate moieties. These are complex carbohydrates linked to the polypeptide backbone via Asn residues at positions 97 and 117 in Ba, 260 and 353 in Bb (Gagnon & Christie 1983; C. M. Anderson, unpublished).

Partial sequence studies of the Bb fragment have previously shown Factor B to be an unusual serine proteinase (Christie *et al.* 1980; Mole & Niemann 1980). The size of the catalytic chain (60000 M_r) is twice that of other known serine proteinases including complement components $C\bar{1}r$, $C\bar{1}s$ and factor $\bar{D}$ (see figure 2 in Reid & Porter 1981). Pancreatic serine proteinases and enzymes of the blood coagulation system all have catalytic chains with molecular mass varying between 25000 and 30000 Da (Young *et al.* 1978; Jackson & Nemerson 1980).

The serine protease domain of Factor B is located in the C-terminal half of fragment Bb, a segment comprising residues 457–739. The amino acid sequence of that segment shows homology to the sequence of the catalytic chain from other known serine proteinases and is compared with bovine thrombin and chymotrypsin A in figure 2. The alignment is in accordance with that proposed in the *Atlas of protein sequence and structure* (Young *et al.* 1978).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

However, to accommodate the longer Bb sequence, seven gaps are inserted in the thrombin and chymotrypsin sequences. Introducing four other breaks in the Bb sequence also increases the homology between the reported alignment of known serine proteinases (Young *et al.* 1978) and the equivalent portion of factor B.

The three amino acid residues essential for catalysis are found at positions 501, 551 and 674, for His, Asp and Ser respectively. They correspond to His-57, Asp-102 and Ser-195 of chymotrypsin. Besides homology detected in the sequence immediately adjacent to these residues, the secondary binding site Ser-Trp-Gly (residues 693–695) is also conserved. This segment of the molecule interacts with a substrate by hydrogen bonds in an anti-parallel β-sheet arrangement.

Several disulphide bridges are usually present in serine proteinases and these include the so-called 'histidine loop', 'methionine loop' and the disulphide bond bringing the primary and secondary binding sites into proximity. In chymotrypsin these are represented by the disulphide bonds linking Cys-42 to Cys-58, Cys-168 to Cys-182 and Cys-191 to Cys-220. In the serine proteinase portion of fragment Bb there are nine cysteine residues. Preliminary evidence indicates that the three conserved disulphide bridges are between Cys-486 and Cys-502, between Cys-631 and Cys-657 and between Cys-670 and Cys-700. One of two other Cys residues (position 571 or 574) is linked to Cys-453 (figure 1) and the other forms a disulphide bond with Cys-590.

Several invariant residues are present in serine proteinases and in addition many amino acids are conserved between members of this enzyme family. When comparing the serine proteinase segment of Bb with thrombin and chymotrypsin (figure 2), approximately 20% and 15% of the aligned residues are identical, respectively.

Although considerable homology exists there are major differences between the structure of the serine proteinase segment of fragment Bb (residues 457–739) and that of other known enzymes of the same family. For instance the length of the sequence containing the catalytic residues varies from 230 residues for chymotrypsin to 283 residues for Factor B. Thrombin with a catalytic chain of 259 amino acid residues was the longest previously known. As a result the spacing between the active site residues varies considerably. The distance between His and Asp is 49 in fragment Bb relative to 44 in chymotrypsin. The spacing between the Asp and the Ser (122 residues) is longer in fragment Bb than that found in chymotrypsin (92 residues). These additional residues make the comparison of the sequences in this region difficult. The differences are better understood from analysis of the genes coding for these molecules (Campbell *et al.* 1984).

More importantly, fragment Bb lacks the usual N-terminal sequence found in other serine proteinase. Crystal structures of chymotrypsinogen, trypsinogen and their active counterparts have shown the N-terminal of the catalytic chain to be essential for maintaining the enzyme in an active conformation (Kraut 1971; Blow 1971; Huber & Bode 1978). In chymotrypsinogen, for example, the substrate binding site which is a pocket near Ser-195 is partially blocked by a segment of the polypeptide chain containing Asp-194. Conversion from zymogen to active enzyme involves a proteolytic cleavage after Arg residue in the sequence Arg-Ile-Val-Asn-Gly. The newly formed α-amino group of Ile (residue 16) then forms an ion pair with Asp-194 and induces a movement of the peptide backbone. This brings Met-192 and Gly-193 from a deeply buried position to the surface of the enzyme, resulting in the opening of the substrate binding site.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Activation of Factor B is by proteolytic cleavage of an Arg-Lys bond (residues 234–235) 222 residues nearer the N-terminus of the molecule. Moreover the sequence Val-Trp-His-Arg (residues 456–460) replaces the sequence Arg-Ile-Val-Asn-Gly found in chymotrypsinogen. Asp-673 however is at a position equivalent to Asp-194 of chymotrypsin (figure 2) but its importance to the activation mechanism is not known at present. This suggests that, whereas factor B is a zymogen with an active site clearly similar to that of other serine proteinases, the activation mechanism is different. A different type of conformational change must occur to make the catalytic site accessible to the substrate. This could be due to the interaction of $C\bar{3}$ with the N-terminal 222 residues domain of Bb (residues 235–456) during activation by factor $\bar{D}$ as proposed by Smith *et al.* (1982). Alternatively activation could involve C3 interaction with part of the serine proteinase domain of Bb (residues 457–739) (Campbell *et al.* 1984). Fragment Bb alone has no C3 convertase activity. Formation of the complex $C\bar{3}\bar{B}$ is essential to the expression of the active site of the convertase and decay of convertase activity is due to dissociation of this complex. Thus the product of the convertase, $C\bar{3}$, is also a component of the C3 cleaving enzyme, resulting in an amplification loop.

	674										
Factor B	D	P	N	T	C	R	G	D	S	G	G	P
C2	D	E	S	P	C	K	G	E	S	G	G	A
Factor D	R	R	D	S	C	K	G	D	S	G	G	P
C1r	K	Q	D	A	C	Q	G	D	S	G	G	V
C1s	E	K	D	S	C	K	G	D	S	G	G	A
trypsin	G	K	D	S	C	Q	G	D	S	G	G	P
chymotrypsin	G	V	S	S	C	M	G	D	S	G	G	P
							195					

FIGURE 3. Sequences around the active site serine. Data is from the following references: Factor D (Johnson *et al.* 1980), C1r (Arlaud & Gagnon 1983), C1s (Carter *et al.* 1983), trypsin and chymotrypsin (Young *et al.* 1978).

The convertase has a trypsin-like activity, cleaving an Arg-Ser bond in C3 (Tack *et al.* 1979; Domdey *et al.* 1982) and after an Arg residue in C5 (Fernandez & Hugli 1978). The specificity of trypsin for basic amino acids is due to the interaction of the substrate with Asp-189 (chymotrypsin numbering) in the binding pocket.

The other serine proteinases of the complement system, also known to have trypsin-like specificity, have an Asp residue at the equivalent position. It is therefore surprising to find an Asn residue (number 668) in the equivalent position (figure 3) although there remains the possibility that Asp-666 fulfils this role.

Although the sequence data clearly establishes that Factor B is a serine proteinase, more information of the three-dimensional structure and interactions within the complex convertase is needed to understand the activation and catalytic mechanism.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

C2

Complement component C2 is a single-chain glycoprotein of approximately 100000 M_r. Activation of C2 is the result of proteolytic cleavage by subcomponent $C\bar{1}\bar{s}$ to give two non-covalently linked polypeptides of 30000 and 70000 M_r. The smaller fragment C2b originates from the N-terminal end of C2 and the larger fragment C2a represents the C-terminal portion. The latter fragment combines with $C\bar{4}$ to generate the classical pathway C3 convertase. The catalytic site of the convertase is in the C2a fragment.

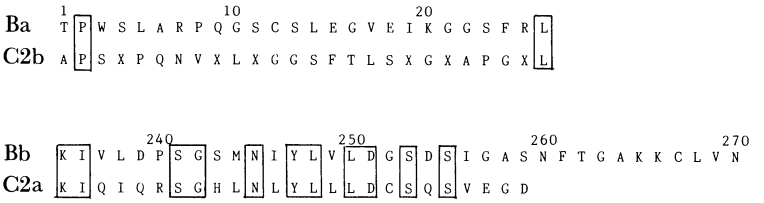

FIGURE 4. Comparison of N-terminal sequences of Ba with C2b and of Bb with C2a. Identical residues are boxed. The single free sulphydril group in Bb is at position 267 and in C2a at position 252 (Factor B numbering).

C2 and Factor B share some functional and structural similarities. Like Factor B, C2 is activated by proteolytic cleavage and the catalytic chain formed is larger than that of other known serine proteinases. Both proteins act as part of large enzyme complexes which, although relatively unstable, cleave the same two substrates, C3 and C5, in an identical manner.

Only limited data is available on the primary structure of C2. Earlier studies have indicated very low homology between the N-terminal sequences of C2b and Ba (Kerr 1979; Kerr & Gagnon 1982) but considerable similarities at the N-terminus of the catalytic chains C2a and Bb (Parkes *et al.* 1983).

The N-terminal sequences of C2a and Bb show 11 identities over a segment of 25 residues. Taking into consideration conservative amino acid replacements (Dayhoff *et al.* 1972) this homology increases to 68%.

Peptides derived from fragments C2b and C2a have recently been studied using procedures adapted from Parkes *et al.* (1983). Briefly these fragments were reduced and alkylated with radiolabelled iodoacetic acid and digested with CNBr. Following separation of the digest by gel-filtration chromatography, radioactive peptides were purified by high pressure liquid chromatography and their amino acid sequence determined.

The N-terminal sequence of six CNBr peptides is presented in figure 5. The alignment of these peptides with the sequence of Factor B is possible because of the high degree of homology between these two proteins.

Three segments of sequence can be aligned. The first segment is located in C2b and shows 49% homology with residues 126–168 of Ba. The second segment, a peptide of 15 residues is equivalent to positions 444–457 of Bb with 33% identities. The third segment covers part of the serine proteinase domain. It consists of four CNBr peptides and the alignment was deduced from the nucleic acid sequence of a cDNA clone for C2 (Bentley & Porter 1984). The amino acid sequence deduced from the sequence of the clone also extended the sequence of the segment to 144 amino acid residues. Comparison with the equivalent sequence of Factor B gives 55 identical positions with 34% homology taking into account gaps introduced to maximize the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

homology. If functionally conserved amino acids are taken into consideration (Dayhoff *et al.* 1972) the homology for these three segments of sequence increases to 58%, 69% and 48% respectively.

Like Factor B, C2 is a serine protease with an unusual activation mechanism. The catalytic chain is larger than usual yet it contains a similar active site, with sequence homology around the Ser residue (figure 3). The residue at position 189 of chymotrypsin is thought to confer substrate specificity. Similarly to Factor B, C2 has trypsin-like specificity and an Asp residue would be expected at the equivalent position, instead of a Ser residue (position 668, Factor B numbering). It could be that specificity is related to Asp-666 (Factor B numbering) as suggested for Factor B. However, remembering that both enzymes function only as part of a large complex, other unknown portions of the convertase could play a role in defining substrate specificity.

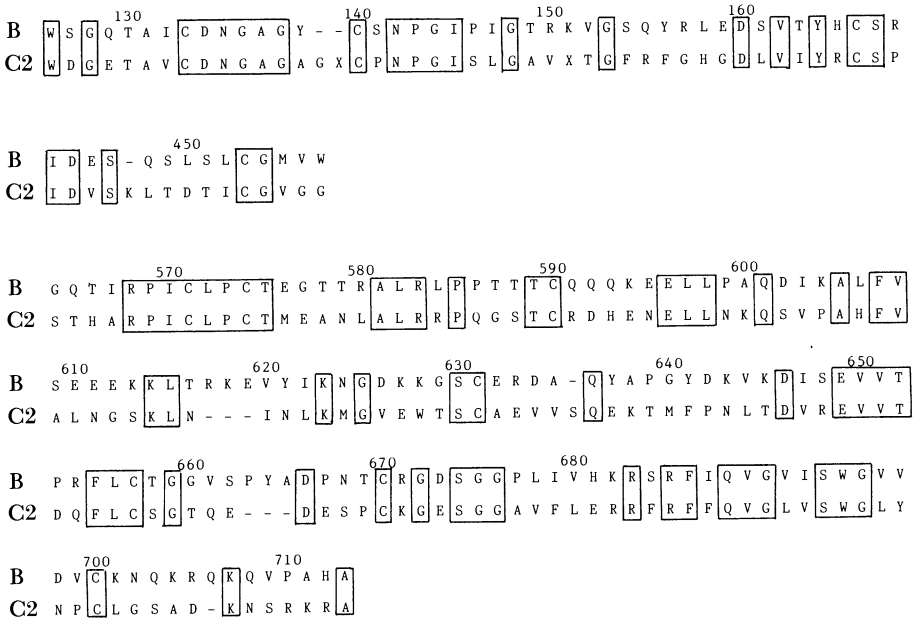

FIGURE 5. Alignment of some CNBr-cleavage peptides of C2 with Factor B. Identical residues are boxed. Gaps (–) have been introduced to maximize homology. Asparagine residues in C2 located at positions 611 and 643 (Factor B numbering) carry carbohydrate moieties.

Complement component C2 and Factor B differ in at least one important aspect. The enzymatic activity of the classical pathway convertase can be enhanced 7–20 fold by treatment of C2 with I_2 (Polley & Muller-Eberhard 1967). It was suggested initially that the enhanced activity was due to oxidation of one or more free sulphydryl groups in C2. This was based on the observation that the reaction of C2 with *p*-chloromercuribenzoate (pCMB) abolishes haemolytic activity whereas prior treatment of C2 with I_2 prevents subsequent inactivation by pCMB, although I_2 is not incorporated in C2.

Factor B and C2 each contains a single free thiol group (Christie & Gagnon 1982; Parkes *et al.* 1983), located close to the N-terminus of fragment Bb and C2a but at different positions and probably in a different environment (figure 4). Reaction with pCMB destroys the haemolytic activity in C2 but not in Factor B. Similarly reaction of I_2 with C2 enhances the activity of C2 but has little effect on Factor B (Parkes *et al.* 1983). The free thiol group in C2

appears to be important in the interaction between the components of the convertase as oxidation leads to increase stability of the complex from a few minutes to several hours. The sulphydryl group in Factor B on the contrary does not seem to be essential to its biological activity.

Comparison of the amino acid sequence of Factor B with the limited data available for C2 indicates that both enzymes share many similarities in their structure and function yet in certain aspects they differ greatly. The convertases of the complement system represent an unusual situation where two complex enzymes, of different composition, perform the same specific function, the activation of C3 and C5.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES