Biochem. J. (1979) **183**, 615–622
Printed in Great Britain

Limited Proteolysis of Complement Components C2 and Factor B

STRUCTURAL ANALOGY AND LIMITED SEQUENCE HOMOLOGY

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(*Received* 21 *March* 1979)

A method is described for the simultaneous purification of milligram quantities of complement components C2 and Factor B. Both products are homogeneous by the criteria of polyacrylamide-gel electrophoresis and *N*-terminal sequence analysis. Component C2 is cleaved by serine proteinase C$\bar{1}$s at an X–Lys bond to give fragment C2a (approx. mol.wt. 74000) and fragment C2b (approx. mol.wt. 34000). The two fragments can be separated by gel filtration without the need for reducing or denaturing agents. Fragment C2b represents the *N*-terminal end of the molecule. Similar results were seen on cleavage of Factor B by Factor D in the presence of component C3. Again two non-covalently linked fragments are formed. The smaller, fragment Ba (approx. mol.wt. 36000), has threonine as the *N*-terminal residue, as does Factor B; the larger, fragment Bb (approx. mol.wt. 58000), has lysine as the *N*-terminal residue. A similar cleavage pattern is obtained on limited proteolysis of Factor B by trypsin, suggesting an Arg–Lys or Lys–Lys bond at the point of cleavage. Although component C2 and Factor B show no apparent *N*-terminal sequence homology, a limited degree of sequence homology is seen around the sites of proteolytic cleavage.

Activation of the third component of complement (C3) is the central event of the complement system. Activation is caused by limited proteolysis effected by one of two complex proteolytic enzymes, the C3 convertases. These enzymes are generated by two separate enzyme cascade systems, the classical and alternative pathways of complement. Although the two convertases show considerable similarity in properties, they have no proteins in common, the classical-pathway C3 convertases being generated from the second (C2) and fourth (C4) components of complement in a Mg^{2+}-dependent reaction catalysed by the serine proteinase C$\bar{1}$s, whereas the alternative-pathway C3 convertase is generated from Factor B and component C3 itself in a Mg^{2+}-dependent reaction catalysed by the serine proteinase Factor D. [For reviews, see Porter & Reid (1979) and Müller-Eberhard (1978)]. Both C3 convertases are believed to cleave C3 at the same site and both exhibit extreme instability, the enzymic activity of the assembled complexes decaying with a half-life of 10–20min at 37°C (Müller-Eberhard *et al.*, 1967; Fearon *et al.*, 1973).

Abbreviations used: the nomenclature of complement components and subcomponents is that recommended by the World Health Organisation (1968); for brevity the descriptors 'component', 'fragment' and 'proteinase' for (e.g.) C2, C2a and C$\bar{1}$s are omitted after their first mention in the text. iPr_2P-F, di-isopropyl phosphorofluoridate.

The enzymically active molecules in these complexes are believed to be C2 and Factor B. Both have been reported to show esterase activity of the same specificity (Cooper, 1975; Cooper & Ziccardi, 1976), and both have been suggested to be serine proteinases (Medicus *et al.*, 1976). The analogous function of the two proteins and their similarity of physicochemical properties, e.g. molecular weight, amino acid composition and susceptibility to proteolysis (Kerr & Porter, 1978) prompted this study of the structural relationship of C2 and Factor B. Such studies are of additional interest, since the structural genes for both proteins are located between the B and D loci in the major histocompatibility complex of chromosome 6 in man (Barnstaple *et al.*, 1979). Characterization of the physiological fragments of limited proteolysis of C2 and Factor B is an important part of our studies on the assembly and function of the classical- and alternative-pathway C3 convertases, which is at present only poorly understood.

Materials and Methods

Materials

The sources of materials used in the purification and characterization of the proteins have been described previously (Kerr & Porter, 1978). Sequencer-grade reagents for the automatic amino acid sequencer were obtained from Pierce-Warriner,

Chester CH1 4EF, U.K., or Rathbone Chemicals, Walkerburn, Peeblesshire, Scotland EH43 6AU, U.K., and benzamidine hydrochloride from Aldrich Chemicals, Gillingham, Dorset SP8 4JL, U.K. Trypsin [1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated] was a product of Worthington Biochemicals.

Methods

Protein purification. C2 and Factor B were purified from outdated human plasma by the method of Kerr & Porter (1978) with several modifications to increase stepwise yields and allow scaling up of the preparation.

(*a*) Euglobulin precipitation was effected by dialysis of human serum (2 litres) brought to pH 5.5 with 1 M-HCl against 8 litres of 5 mM-EDTA (pH 5.5)/0.5% (w/v) benzamidine hydrochloride.

(*b*) For the column chromatography on CM-Sephadex CM-50, all buffers were made 0.5% (w/v) benzamidine hydrochloride and the iPr_2P-F omitted.

(*c*) For the final purification steps for C2, the DEAE-Sephadex A25 column was replaced by one of DEAE-Sepharose equilibrated in 5 mM-veronal buffer, pH 8.5, containing 0.5 mM-$CaCl_2$, 2.0 mM-$MgCl_2$ and 40 mM-NaCl, and developed with a linear NaCl gradient from 40 to 80 mM. All C2-active fractions were pooled and final traces of Factor B removed by passage through a column (10 cm × 2.0 cm) containing Sepharose 4B-bound anti-(Factor B) antibody.

(*d*) For the final purification of Factor B, the DEAE-Sephadex A25 column was replaced by one of DEAE-Sepharose equilibrated in 5 mM-veronal buffer, pH 8.5, containing 0.5 mM-$CaCl_2$, 2.0 mM-$MgCl_2$ and 40 mM-NaCl, and developed with a linear NaCl gradient from 40 to 100 mM. Active fractions were pooled and the small traces of contaminant protein removed by chromatography on a column (15 cm × 3.0 cm) containing 'aged' CNBr-activated Sepharose 4B (Kerr & Porter, 1978) equilibrated in 5 mM-veronal buffer, pH 8.5, containing 0.5 mM-$CaCl_2$, 2.0 mM-$MgCl_2$ and 40 mM-NaCl. Under these conditions the contaminant protein, believed to be haemopexin, passes straight through, and the Factor B, which is retarded, can be eluted by extensive washing with the same buffer, or, better, by a small volume of 0.1 M-sodium phosphate, pH 6.0.

C4 and $C\bar{1}s$ were purified by the methods of Gigli *et al.* (1976, 1977). C3 was purified by the method of Tack & Prahl (1976) as modified by Goers & Porter (1978).

Other methods

Automated *N*-terminal amino acid-sequence analysis was done in a Beckman 890C sequencer with 1.0 M-Quadrol [1,1′,1″,1‴-(ethane-1,2-diyldinitrito)-tetrakis(propan-2-ol)] trifluoroacetate, pH 9.5 (Beckman Instruments, Palo Alto, CA, U.S.A.) as described by Brauer *et al.* (1975). Thiazolinones released were converted into phenylthiohydantoins, which were identified by t.l.c. or by amino acid analysis after back-hydrolysis with HI as described by Reid (1976) or by high-pressure liquid chromatography on a Bondapak C-18 column (Bridgen *et al.*, 1976) with a Waters Associates (Northwich, Cheshire, U.K.) instrument.

Amino acid analyses were carried out by using a Durrum D500 analyser. All other methods were as described previously (Kerr & Porter, 1978).

Results

Purification of C2 and Factor B

It was previously reported that, during the development of a purification scheme for C2 (Kerr & Porter, 1978), we identified a major contaminant that co-purified with C2 as complement Factor B. We have now extended these studies to show that, with only minor modifications to the method, both C2 and Factor B can be isolated in good yield from the same serum. By the addition of benzamidine to the buffers in the early stages of the purification and by the replacement of the DEAE-Sephadex by a DEAE-Sepharose column, which allows a more rapid and efficient removal of small traces of $C\bar{1}s$ from the preparation, yields of C2 were increased to over 20%, so that 8–10 mg of C2 could be purified from 2 litres of serum. The Factor B (80–100 mg), which was homogeneous by the criteria of sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and *N*-terminal amino acid analysis, represents a yield of over 20% from the starting serum and therefore compares favourably with other published purification schemes (Curman *et al.*, 1977; Hunsicker *et al.*, 1973).

Limited proteolysis of C2 by $C\bar{1}s$

Incubation of highly purified C2 with catalytic amounts of $C\bar{1}s$ leads to the loss of haemolytic activity. This loss of activity is the result of cleavage of the single polypeptide chain of C2 (apparent mol.wt. 104000) into two fragments, C2a and C2b. The two fragments are clearly visible on sodium dodecyl sulphate/polyacrylamide gels run under reducing or non-reducing conditions, and therefore are not covalently linked (Kerr & Porter, 1978; Nagasawa & Stroud, 1977). The smaller fragment characteristically is seen as a doublet on gels run as described by Laemmli (1970), the mobility of both bands of this doublet being decreased in gels run under reducing conditions. From the mobility of fragments C2a and C2b on gels of several acrylamide concentrations run under reducing conditions, apparent mol.wts. of 74000 and 34000 respectively were estimated.

Digestion of milligram quantities of C2 by C1s at an enzyme/substrate ratio of 1:100 for up to 30 min gave no products identifiable by sodium dodecyl

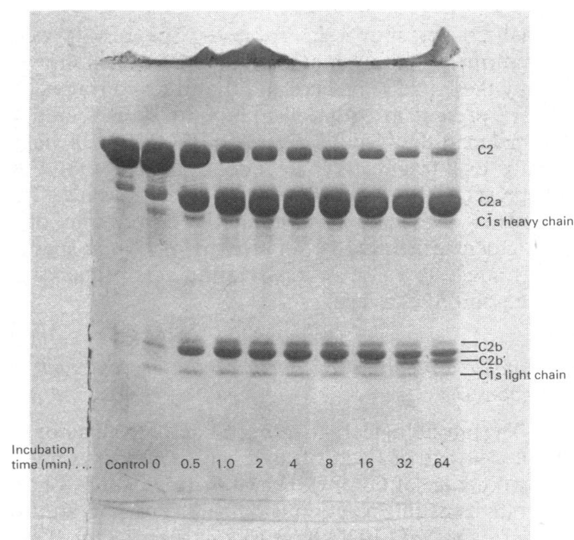

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide gels [run as described by Laemmli (1970)] of samples taken at different times during a digest of C2 by C1̄s*

C2 (0.8 mg) in 1.0 ml of 5 mM-veronal buffer (pH 8.5)/ 0.5 mM-$CaCl_2$/2.0 mM-$MgCl_2$/40 mM-NaCl was incubated with C1̄s (8 μg) at 37°C. Samples (20 μl) were removed at different times and incubated with 20 μl of 10 mM-dithiothreitol/8 M-urea/2% (w/v) sodium dodecyl sulphate/0.2 M-Tris/HCl (pH 8.0) at 37°C for 30 min before loading on the gel.

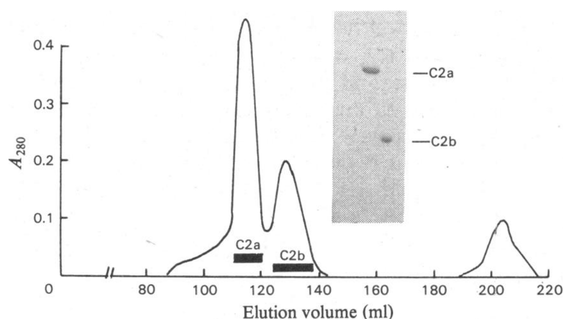

Fig. 2. *Chromatography of a limited digest of C2 by C1̄s on Sephadex G-100*

C2 (8.6 mg) in 10 ml of 5 mM-veronal buffer (pH 8.5)/ 0.5 mM-$CaCl_2$/2.0 mM-$MgCl_2$/40 mM-NaCl was incubated with C1̄s (15 μg) at 37°C. After 60 min the reaction was stopped by the addition of 10 mM-iPr_2P-F and the products chromatographed on a column (220 cm × 3.3 cm) of Sephadex G-100 equilibrated in 0.1 M-$NaHCO_3$. The insert shows sodium dodecyl sulphate/polyacrylamide gels of samples of the two pools A and B. The absorbance of the third peak is due to the veronal buffer.

sulphate/polyacrylamide-gel electrophoresis other than C2a and C2b (Fig. 1). Since both bands in the C2b doublet appear simultaneously and remain of the same relative intensity, it is most likely that this is due to charge heterogeneity rather than to two differently sized polypeptide chains.

This interpretation is consistent with the results of *N*-terminal amino acid analyses of the digest carried out after 5 and 10 min, which showed the appearance of only one new *N*-terminal amino acid, namely lysine. The quantity of this amino acid increased with time and was present after 10 min in approximately equimolar amounts with alanine, the *N*-terminal amino acid of intact C2.

Upon extended digestion of C2 by C1̄s, other sites of cleavage could be detected. C2b was slowly cleaved to give a smaller fragment of apparent mol. wt. 32000. This was eventually digested further to give two smaller disulphide-linked fragments of apparent mol.wts. 21000 and 11000. It should, however, be stressed that these minor cleavages are unlikely to be important in the activation or decay of the C3 convertase, since under similar conditions the activation and decay curve is complete in less than 30 min, and the products of C2 cleavage in the presence of C4 appear to be C2a and C2b, identical with those produced in the absence of C4 (M. A. Kerr, unpublished work). The relationship of these smaller fragments to the physiologically active C2 kinin peptides (Donaldson *et al.*, 1977) has not been investigated.

Isolation and characterization of C2a and C2b

C2a and C2b are not covalently linked and can be separated easily by gel filtration on Sephadex G-100 without the need for denaturing reagents (Fig. 2). The 60 min digest of C2 by C1̄s (enzyme/substrate ratio 1:600) showed only the two fragments, and attempts to detect minor fragments by amino acid analysis were unsuccessful. Amino acid analyses of C2a and C2b showed them to be present in equimolar amounts.

The full amino acid analyses of C2a and C2b are given in Table 1. C2a, as would be expected from its acidic behaviour on electrophoresis, is rich in aspartic and glutamic acids, though no attempt was made to assess the degree of amidation of the residues. C2b has a most unusual amino acid composition, being very rich in the small uncharged amino acids, glycine (13.4%) proline (9.8%) and cysteine (5.3%), but despite the high proline and glycine content, no hydroxylysine or hydroxyproline were detected, suggesting that no collagen-like sequences such as those found in C1q are present.

N-Terminal analysis of isolated C2b showed alanine, the same *N*-terminal residue as that of intact C2. Two attempts to determine the *N*-terminal amino acid of C2a were unsuccessful, although the dansyla-

tions were carried out at the same time as that of a total digest of C2, which showed two amino acids, namely alanine and lysine. The reason for this is unclear, since automatic Edman degradation carried out on C2a proved conclusively that lysine is the *N*-terminal; but it may explain the report of Nagasawa & Stroud (1977) that alanine was the *N*-terminal of C2, C2a and C2b.

The results of automatic amino acid-sequence analysis are shown in Table 2. The results were confirmed by analysis of a digest of C2, which showed only these two sequences and that the two fragments were present in equimolar amounts. It is therefore concluded that limited digestion of C2 by C$\bar{1}$s yields only two fragments, C2a and C2b, and that C2b represents the *N*-terminal part of a molecule. The unusual presence of lysine at the *N*-terminus of a fragment generated by an enzyme of tryptic specificity suggests a double basic-amino acid sequence at the point of cleavage.

Table 1. *Amino acid composition of C2, C2a and C2b*

Amino acid	Amino acid composition (residues/100 residues)		
	C2	C2a	C2b
Cys	3.2	2.7	5.3
Asp	11.2	12.7	8.4
Thr	5.0	4.3	4.9
Ser	7.9	7.3	8.7
Glu	10.4	10.5	8.7
Pro	5.7	4.6	9.8
Gly	9.2	6.6	13.4
Ala	6.3	6.2	6.5
Val	6.8	6.7	7.0
Met	1.9	2.7	0.8
Ile	4.2	4.9	2.9
Leu	9.4	10.3	6.3
Tyr	2.7	1.6	3.4
Phe	4.4	4.4	4.0
His	2.7	2.8	2.1
Lys	5.1	5.9	2.5
Arg	5.3	5.5	5.3
Trp	ND*	ND	ND

* Abbreviation used: ND, not determined.

Limited proteolysis of Factor B by trypsin and by Factor D

The physiological activator of Factor B is Factor D, but cleavage of Factor B by Factor D occurs only in the presence of C3 or C3b. and therefore study of the products of this reaction is difficult. Previous studies (Curman *et al.*, 1977) have shown that Factor B can be cleaved in an analogous manner by trypsin and this cleavage is not dependent on the presence of C3 or C3b. If, however, the reaction is carried out in the presence of C3b, an active C3 convertase can be assembled, suggesting that the process is at least qualitatively similar to the cleavage by Factor D (Brade *et al.*, 1974).

Sodium dodecyl sulphate/polyacrylamide gels corresponding to a time course for the digestion of Factor B by trypsin (substrate/enzyme molar ratio 80:1) and by Factor D in the presence of C3 (B/C3/D ratio 100:100:1) are shown in Fig. 3. Our results show that Factor B is cleaved by Factor D at a single point to yield two fragments, Ba (app. mol.wt. 36000) and Bb (app. mol.wt. 58000). The two fragments are seen on gels run under non-reducing

Table 2. *Amino acid-sequence analysis of the N-terminal sequence of C2a and C2b*

Residue no.	Method of identification of amino acid phenylthiohydantoin derivative ...	Sequence: C2b H.p.l.c.*	Sequence: C2b Amino acid analysis	Sequence: C2a H.p.l.c.	Sequence: C2a Amino acid analysis	Proposed sequence C2b	Proposed sequence C2a
1		Ala		Lys	Lys	Ala	Lys
2		Pro		Ile	Ile	Pro	Ile
3		Ser		Gln		Ser	Gln
4		—		Ile		X	Ile
5		Pro		Gln		Pro	Gln
6		Glu		Ser		Glu	Ser
7		Asn		—		Asn	—
8		Val/Met	Val	Val		Val	Val
9		—		—		X	—
10		Leu		Lys		Leu	Lys

* Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

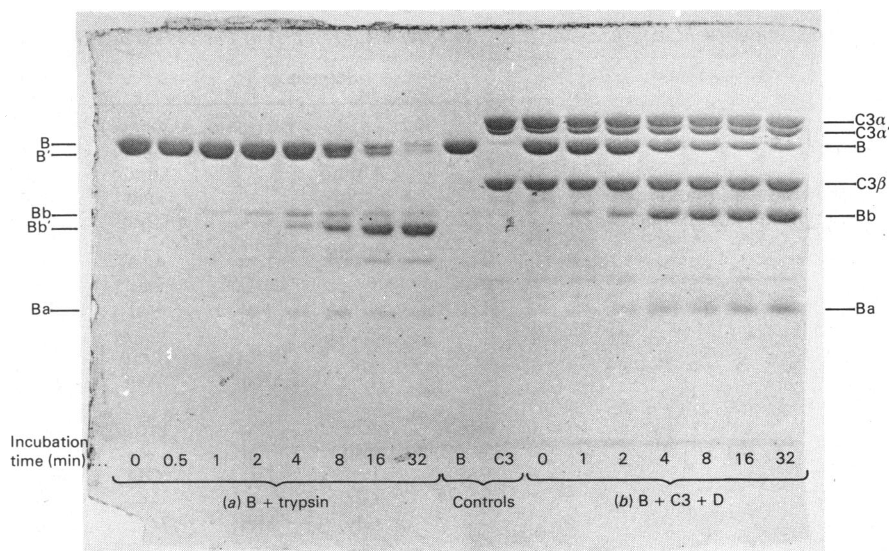

Fig. 3. *Sodium dodecyl sulphate/polyacrylamide gels [run as described by Laemmli (1970)] of samples taken at different times during a digest of Factor B by (a) trypsin and (b) Factor D in the presence of C3*

Factor B (0.25mg) in 1.0ml of 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl was incubated with (*a*) trypsin (1 µg) or (*b*) C3 (0.5 mg) and Factor D (10 µg). Samples were removed at different times and incubated with 20 µl of 10mM-dithiothreitol/8M-urea/2% (w/v) sodium dodecyl sulphate/0.2M-Tris/HCl (pH 8.0) at 37°C for 30min before loading on to the gel.

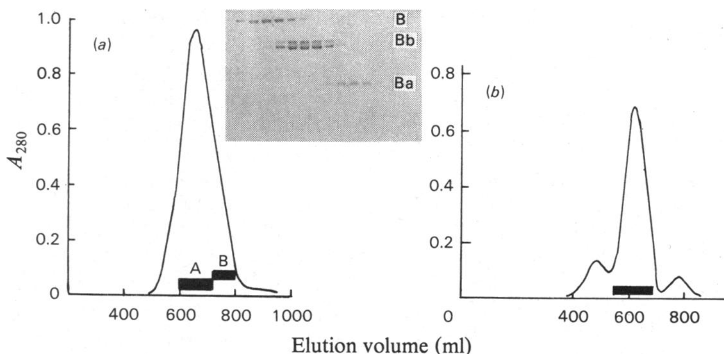

Fig. 4. *Chromatography of a limited tryptic digest of Factor B on Sephadex G-100*

(*a*) Factor B (25 mg) in 10ml of 5mM-veronal buffer (pH 8.5)/0.5mM-$CaCl_2$/2.0mM-$MgCl_2$/40mM-NaCl was digested with trypsin (100 µg) for 15 min. The reaction was stopped by the addition of 1 mM-iPr_2P-F and the products chromatographed on a column (220cm × 3.3cm) of Sephadex G-100 equilibrated in 0.1M-$NaHCO_3$. (*b*) Rechromatography of pool A from the column under identical conditions. The insert shows sodium dodecyl sulphate/polyacrylamide gels of samples taken from every other (10ml) fraction across the protein peak (52–74).

conditions and are therefore not linked by disulphide bonds. Factor B is, however, cleaved by trypsin at several positions to give a cleavage pattern more complex than had been suggested by previous studies (Curman *et al.*, 1977). The first cleavage apparently results in the release of a small peptide from the Factor B to yield a species we have termed B'. The subsequent characteristic cleavage of Factor B to Ba and Bb as a result yields a species Bb' smaller than that derived from the cleavage by Factor D. Fragment Ba derived by either cleavage method showed similar mobility on sodium dodecyl sulphate/polyacrylamide gels. Upon extended incubation with trypsin, fragment Ba was completely

Table 3. *Amino acid sequence analysis of the N-terminal residues of Factors Ba and Bb*

Residue no.	Method of identification of amino acid phenylthiohydantoin derivative ...	Sequence: Ba T.l.c.	Sequence: Ba Amino acid analysis	Sequence: Bb (major sequence) T.l.c.	Sequence: Bb (major sequence) Amino acid analysis	Proposed sequence Ba	Proposed sequence Bb
1		Thr	Abu*	Lys	Lys	Thr	Lys
2		Pro	Pro	Ile/Leu	Ile	Pro	Ile
3		Trp	Gly Ala	Val	Val	Trp	Val
4		—	Ala	Ile/Leu	Leu	(Ser)	Leu
5		Leu Ile	Leu	Asp	Asp	Leu	Asp
6		Ala	Ala	Pro	Pro	Ala	Pro
7		—	Glu	Thr	—	(Gln)	Thr
8		Pro	Pro	Gly	Gly	Pro	Gly
9		—	Glu	—	—	Glx	—
10		—	Gly	Met		Gly	Met

* Abbreviation used: Abu, 2-aminobutyric acid.

destroyed. Nevertheless, by selection of a limited time of digestion and a low enzyme/substrate ratio we were able to obtain fragments suitable for the present comparative study. Such a digest also represents a convenient starting point for a more extensive characterization of the molecule.

Characterization of the trypsin-derived fragments of Factor B

The results of chromatography of a limited tryptic digest of Factor B on Sephadex G-100 are shown in Fig. 4. Rechromatography of the middle fractions of the single protein peak that emerged allowed resolution of the digest into two identifiable pools, the first corresponding to a mixture of Bb and Bb′, the second to Ba. *N*-Terminal amino acid analysis of the isolated pools showed a single *N*-terminal threonine for the Ba fragment, which is the same as that of the whole molecule (Kerr & Porter, 1978). Analysis of the Bb pool showed two *N*-terminal residues, namely lysine and isoleucine, present in approx. 2:1 ratio.

N-Terminal amino acid analysis of Factor B digested by Factor D in the presence of C3b showed the appearance of a single new *N*-terminal lysine residue and therefore, in view of the similarity in size of the trypsin- and Factor D-derived fragments of Factor B and the unusual *N*-terminal lysine residue generated in both cases, it is highly likely that both enzymes cleave Factor B at the same site. The appearance of an *N*-terminal lysine residue from a tryptic digest further suggests that this site of cleavage involves an Arg–Lys or Lys–Lys bond.

The results of automated Edman degradation of the isolated tryptic fragments Ba and Bb are shown in Table 3. The *N*-terminal amino acid sequence of Factor B was identical with that of fragment Ba for all amino acids identified up to residue 10. The Bb fragment ran as a double sequence; the major sequence beginning with lysine represented 70% of the material. The minor sequence beginning with isoleucine was the same sequence displaced by one amino acid.

Since it is difficult to envisage that this lack of a single amino acid could account for the difference in mobility of the two species in the Bb pool on sodium dodecyl sulphate/polyacrylamide gels, it must be assumed that the first point of cleavage of Factor B by trypsin is in the *C*-terminal part of the molecule and therefore in the *C*-terminal part of Bb.

Discussion

The similarity of C2 and Factor B in function and in physiological properties is well documented. The similarity extends to a similarity of amino acid composition, although the proteins show no antigenic cross-reactivity (Kerr & Porter, 1978). Our present results show the two proteins to be similar also in their susceptibility to limited proteolysis and to exhibit a limited degree of sequence homology.

The extreme susceptibility to proteolytic enzymes has hampered the purification of both proteins, in particular C2. This proteolysis is, however, in both cases very limited, involving in the main cleavage at a single site. It is apparent that, *in vivo*, this single

Table 4. *Amino acid sequences of the fragments of limited proteolysis of C2 and Factor B*

Residue ...	1	2	3	4	5	6	7	8	9	10
C2a	(Lys/Arg)	Lys	Ile	Gln	Ile	Gln	Ser	X	Val	X	Lys
Bb	(Lys/Arg)	Lys	Ile	Val	Leu	Asp	Pro	Thr	Gly	X	Met
C2b		Ala	Pro	Ser	X	Pro	Glu	Asn	Val	X	Leu
Ba		Thr	Pro	Trp	Ser	Leu	Ala	Gln	Pro	Glx	Gly

cleavage is responsible for the generation and decay of the C3-cleaving activity when the C2 or Factor B is part of the C3 convertase complex. The similar pattern of cleavage suggests a similarity in the overall structure of the two proteins. In both cases the smaller of the two fragments generated represents approximately one-third of the size of the intact molecule and is derived from the *N*-terminal part of the molecule. The subsequent ease of the separation of the two fragments in each case suggests that both proteins comprise two separate domains linked by a single stretch of amino acid sequence highly vulnerable to proteolysis. In spite of the similarity of properties of the two proteins and the close linkage of their structural genes, C2 and Factor B have shown little amino acid-sequence homology in the small amount of sequence that has been determined. Of the first ten residues of C2b and Ba, only one is identical; of the first ten residues of C2a and Bb, two are identical (Table 4). There is, however, most similarity around the sites of cleavage of C2 and Factor B and this may reflect a similarity of sequence in the two proteins around other functionally important sites. No similarity of sequence C2 or Factor B with C4 (Gigli *et al.*, 1977) or with other products of the genes of the major histocompatibility complex has been observed.

The serine-proteinase nature of C2 and Factor B

It has been shown by Cooper (1971, 1975) that preparations of C2 and Factor B were able to hydrolyse certain synthetic esters containing basic amino acids. The esterolytic activity of C2 was increased slightly upon cleavage of the molecule by C$\bar{1}$s, and the system therefore showed some characteristics of zymogen–enzyme transformation, although the increase in activity is only 1.5–2-fold compared with the 10^6-fold seen, for example, for the trypsinogen–trypsin conversion (Kerr *et al.*, 1975). Although previous studies had suggested that both C2 and Factor B were not sensitive to the serine-proteinase inhibitor iPr_2P-F (Cooper, 1975; Polley & Müller-Eberhard, 1968), Medicus *et al.* (1976) have demonstrated inhibition of Factor B and C2 haemolytic activity by the reagent. However, the proteins show considerable differences in molecular properties from those of other serine proteinases. For example, the proposed active site containing polypeptide chains C2a and Bb has a molecular weight far in excess of that found for all other serine proteinases of the digestive, blood-clotting or complement system, which have mol.wts. of 25000–30000. In addition, although our results now show C2a and Bb to be the *C*-terminal portion of the molecule, which is presumably a prerequisite for a zymogen–enzyme transformation (Neurath & Walsh, 1977), the *N*-terminal sequence shows no similarity to the highly conserved and functionally important sequences of other serine proteinases. The presence of an Ile-Val-X-Gly, Ile-Ile-X-Gly or Val-Val-X-Gly sequence at the *N*-terminus of the enzymically active chain is essential, since the two hydrophobic residues cause reorientation of this part of the polypeptide chain such that both are buried deep in the protein where the *N*-terminal amino acid forms a salt bridge with aspartic acid-194 (chymotrypsin numbering), which allows formation of the substrate-binding site (Kraut, 1971). If C2 and Factor B are indeed serine proteinases, it appears that they must represent either the members of a separate group of enzymes only distantly or not at all related to the other serine proteinases, or a new mechanism of activation of the zymogens of serine proteinases.

I thank Professor R. R. Porter for his advice throughout this work, Mr. A. C. Willis for his excellent technical assistance and Dr. J. Gagnon and Mr. T. Gascoyne for assistance in amino acid-sequence determination and amino acid analyses. I am a Wellcome Senior Research Fellow.

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