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Large-scale isolation of complement receptor type 1 (CR1) from human erythrocytes

Proteolytic fragmentation studies

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A large-scale procedure for the isolation of complement receptor type 1 (CR1, the C3b receptor) from human erythrocytes is described. Two of the four known phenotypes of CR1 are detectable in the isolated material. Amino acid and hexosamine analysis of the A phenotype (M_r 240000) indicates a polypeptide chain length of about 2030 amino acids and a carbohydrate content of 8%. Both *N*- and *O*-linked sugars appear to be present. Trypsin digestion of isolated CR1 shows that it is degraded rapidly and extensively, and no stable products of M_r greater than 25000 are found. The ability of the receptor to bind to solid-phase ligand is destroyed after a single cleavage by trypsin. The capacity of the receptor to act as a cofactor for Factor I-mediated cleavage of soluble C3b is, however, only gradually decreased by proteolysis, and 30% of this activity remains after extensive degradation. The same pattern of loss of binding to solid-phase ligand, with partial retention of interaction with soluble ligand, is also characteristic of the complement proteins Factor H and C4bp, which are functionally related to CR1.

INTRODUCTION

Complement receptor type 1 (CR1) was first isolated from human erythrocytes by Fearon (1979, 1980) and was described as a glycoprotein of M_r 205000–250000. It is also present on monocytes, macrophages, B lymphocytes and some T lymphocytes, polymorphonuclear leucocytes (Fearon, 1980), kidney podocytes (Kazatchkine *et al.*, 1982) and possibly in other tissue sites (Nyland *et al.*, 1979). This receptor binds particles bearing the C3b or (weakly) the iC3b fragment of complement component C3, and also the C4b fragment of complement component C4 (for review see Fearon & Wong, 1983). It serves as a cofactor for the Factor I-mediated proteolytic degradation of C3b to iC3b, or of C4b to C4c+C4d (Fearon, 1979; Iida & Nussenzweig, 1981). In this reaction, it is thought that C3b or C4b first binds to the cofactor protein, forming a 1:1 molecular complex, and C3b or C4b in this complex is degraded by the proteinase Factor I. This Factor I-cofactor activity is also a property of the abundant serum complement proteins Factor H (M_r 155000) and C4bp (M_r about 500000). Comparisons of the cofactor activities of CR1, Factor H and C4bp have been summarized before (Sim & Sim, 1983).

The structural gene loci for CR1, Factor H and C4bp are closely linked (Rodriguez de Cordoba *et al.*, 1984; Lublin *et al.*, 1984), and it is likely, despite their differences in gross size, that these proteins exhibit extensive structural similarities. CR1 is polymorphic in size, in that four co-dominantly expressed allotypic variants of apparent M_r values between 160000 and 250000 have been described (Dykman *et al.*, 1983, 1984; Wong *et al.*, 1983; Holers *et al.*, 1984). These variants have been named (Holers *et al.*, 1984) type A (gene frequency 0.83; apparent M_r 190000), type B (0.16; M_r 220000), type C (0.01; M_r 160000) and type D (< 0.01; M_r 250000). The apparent M_r values for these variants are estimated from SDS/polyacrylamide-gel electrophoresis under non-reducing conditions.

CR1 has been purified on a small scale in several laboratories, by using methods based on that of Fearon (1979) (see, e.g., Iida & Nussenzweig, 1981; Daha *et al.*, 1984; Sim & Sim, 1983). A larger-scale preparation, yielding 1.5 mg of CR1 from 10^{14} erythrocytes, has been mentioned (Dobson *et al.*, 1981). Atkinson and colleagues have been successful in applying small-scale ligand and antibody affinity chromatography techniques to isolate CR1 in analytical quantities from erythrocytes and leucocytes (Dykman *et al.*, 1983, 1984) and have also referred to a larger-scale isolation by using a monoclonal antibody affinity column (Holers *et al.*, 1984).

To facilitate structural studies on CR1, a simplified large-scale isolation method has been developed, which does not require the use of fresh blood donations. This method is described below.

MATERIALS AND METHODS

Commercial materials

Proteinase inhibitors, namely di-isopropyl phosphorofluoridate, phenylmethanesulphonyl fluoride, 1,10-phenanthroline and soya-bean trypsin inhibitor (type I-S) were from Sigma Chemical Co., Poole, Dorset, U.K. Trypsin [L-1-chloro-4-phenyl-3-tosylamidobutan-2-one-('TPCK')-treated] was from Worthington Diagnostics, Freehold, NJ, U.S.A. Iodoacetamide was from BDH Chemicals, Poole, Dorset, U.K., and benzamidine was from Aldrich Chemical Co., Poole, Dorset, U.K. Non-ionic detergents Nonidet P40 and Triton X-100

Abbreviations used: SDS, sodium dodecyl sulphate; CR1, complement receptor type 1; C3u, the inactive form of complement component C3, in which the thioester is cleaved (Parkes *et al.*, 1981) [also termed C3i or C3 (H_2O) (Müller-Eberhard, 1980)].

(both octylphenoxypolyethoxyethanols) and Emulphogene BC-720 (polyoxyethylene 10-tridecyl ether) were from Sigma Chemical Co. DEAE-Sephacel and CNBr-activated Sepharose 4B were from Pharmacia, Milton Keynes, Bucks., U.K., and DEAE-cellulose (DE 52) was from Whatman, Maidstone, Kent, U.K.

Proteins and affinity adsorbents

C3 was prepared and converted into the inactive form C3u in which the thiolester bond is cleaved as summarized previously (Micklem *et al.*, 1984). C3u was equilibrated in 100 mM-sodium borate buffer, pH 8.3, and coupled to CNBr-activated Sepharose 4B at a final density of 7.5 mg of C3u/ml of packed Sepharose. Factors H and I were isolated as described by Hsiung *et al.* (1982) and Sim & DiScipio (1982) respectively.

Buffers

Buffers described in the protein purification procedures listed below were made up of 4.57 mM-Na_2HPO_4/0.43 mM-NaH_2PO_4, pH 7.8 ('5 mM-sodium phosphate') or 6.1 mM-K_2HPO_4/3.9 mM-KH_2PO_4, pH 7.0 ('10 mM-potassium phosphate'), to which were added chelating agents, proteinase inhibitors or detergents. After these additions to the buffers, the pH was re-adjusted, as appropriate, to 7.8 with 0.1 M-H_3PO_4 or 0.1 M-NaOH, or to 7.0 with 0.1 M-H_3PO_4 or 0.1 M-KOH.

CR1 preparation

Erythrocytes. Erythrocytes were prepared from 20–40 units of plasma-reduced blood (corresponding to 4.0–8.4 litres of settled erythrocytes), kindly supplied by the Oxford Regional Blood Transfusion Service, John Radcliffe Hospital, Oxford, U.K. The plasma-reduced blood was used 10–21 days after donation. The pooled cells were washed by repeated centrifugation (3000 *g* for 30 min) in 40 litres of 0.15 M-NaCl. The buffy coat was removed carefully after each centrifugation, and the cells were then filtered through nylon-wool wadding (Cellselect Leukocyte Filters; Lorne Laboratories, Maidenhead, Berks., U.K.) to remove leucocytes. After filtration the cells were again washed with 40 litres of 0.15 M-NaCl and adjusted to a volume of 9 litres. All above and subsequent steps were done at 4 °C unless otherwise stated.

Erythocyte membranes. The erythrocytes were lysed in the presence of proteinase inhibitors by dilution with 5 mM-sodium phosphate/0.5 mM-EDTA, pH 7.8, to a total volume of 20 litres. Proteinase inhibitors were added immediately before lysis to provide final concentrations in the 20-litre suspension as follows: phenylmethanesulphonyl fluoride: 1 mM; di-isopropyl phosphorofluoridate, 0.625 mM; 1,10-phenanthroline, 0.01 mM; iodoacetamide, 1.4 mM; soya-bean trypsin inhibitor, 12.5 mg/l; benzamidine, 5 mM. The ghosts were then washed with 75 litres of 5 mM-sodium phosphate/0.5 mM-EDTA/1 mM-benzamidine/0.5 mM-phenylmethanesulphonyl fluoride, pH 7.8, by using tangential-flow filtration in a standard Pellicon cassette system (Millipore, Harrow, Middx., U.K.) equipped with two Durapore 0.5 μm-pore-size filter cassettes (Millipore). The ghosts were finally concentrated to a slurry of total volume 2.0–3.0 litres in the same apparatus. The erythrocyte-ghost slurry was stored frozen at −70 °C.

Detergent solubilization. Thawed erythrocyte membranes were selectively extracted with non-ionic detergent as described previously (Sim & Sim, 1983). Briefly, solid NaCl was added to the ghost slurry to provide a final concentration (after the additions listed below) of 0.15 M-NaCl, and proteinase inhibitors were added to the same concentrations as at the erythrocyte lysis step. The preparation was then made 1% (w/v) Nonidet P40 and 1% (w/v) Triton X-100 by addition of appropriate volumes of stock 20% (w/v) solutions of these detergents, and incubated at 37 °C for 40 min. Insoluble residue was pelleted by centrifugation at 100000 *g* for 1 h.

Ion-exchange chromatography. The detergent-solubilized material (2.2–3.3 litres) was dialysed three times to equilibrium against 5 vol. of 0.1% (w/v) Emulphogene/1 mM-benzamidine/HCl, pH 7.0, and then once against 5 vol. of 5 mM-phosphate/0.5 mM-EDTA/5 mM-benzamidine, pH 7.8. The material was applied to a column (85 cm × 5 cm diam.) of DEAE-Sephacel and DEAE-cellulose (DE 52) mixed 1:1 (v/v), equilibrated in 5 mM-sodium phosphate/0.5 mM-EDTA/5 mM-benzamidine, pH 7.8. Flow rate was 250–300 ml/h. The column was washed with 4 litres of the starting buffer also containing 0.5 mM-phenylmethanesulphonyl fluoride, then eluted with a linear gradient (8 litres) of 0–0.2 M-KCl in the starting buffer. CR1 was located in the gradient elution by SDS/polyacrylamide-gel electrophoresis of fractions and by assay of cofactor activity. The column was finally washed with 2 litres of the same buffer containing 0.5 M-KCl.

Affinity chromatography. Fractions containing CR1 were pooled and made 0.25 mM with respect to di-isopropyl phosphorofluoridate, and dialysed against 6 vol. of 10 mM-potassium phosphate/0.5 mM-EDTA/5 mM-benzamidine/0.1% (w/v) Emulphogene, pH 7.0, and applied to a column (20 cm × 1.5 cm diam.) of C3u–Sepharose equilibrated in the same buffer, at a flow rate of 50 ml/h. The column was washed with 200 ml of the starting buffer, and CR1 was then eluted with a 300 ml linear gradient of 0–0.35 M-NaCl in the same buffer. Fractions containing CR1 were identified as above, and pooled. This material was then re-run and re-eluted from the same C3u–Sepharose column under the conditions described above.

The isolated CR1 was stored at −20 °C.

Assay of CR1

The Factor I-cofactor activity of CR1 was measured by determining the extent or rate of degradation of ^{125}I-labelled C3b to iC3b in the presence of pure Factor I and a source of CR1, as previously described (Sim & Sim, 1983). Control assays were done in the absence of Factor I, to eliminate possible interference from proteolytic activity not attributable to Factor I.

SDS/polyacrylamide-gel electrophoresis

The SDS/polyacrylamide-gel electrophoresis system of Laemmli (1970) was used, with a running gel of 6.5% (w/v) polyacrylamide and a stacking gel of 3% (w/v) polyacrylamide. Samples for electrophoresis were prepared, and gels were stained with Coomassie Blue and destained as described by Fairbanks *et al.* (1971). Silver staining was done as described by Wray *et al.* (1981). M_r standards for SDS/polyacrylamide-gel electrophoresis

(myosin, phosphorylase *b*, catalase, ovalbumin and carboxypeptidase) were purchased from Sigma Chemical Co.

Amino acid and hexosamine analyses

Amino acid composition was determined by duplicate 24 h, 48 h and 72 h HCl hydrolyses, hexosamines by 24 h toluene-*p*-sulphonic acid digestion, and tryptophan by spectrophotometric methods. These analyses were performed after complete reduction of CR1 and alkylation with iodoacetic acid. Methods used were as described previously (Sim & DiScipio, 1982).

Protein assay

Protein was assayed by amino acid analysis or by a modification of the Lowry method (Maddy & Spooner, 1970), with bovine serum albumin as standard.

Radioiodination

C3b and CR1 were labelled with carrier-free [^{125}I]iodide (Amersham International, Amersham, Bucks., U.K.) by the iodogen method (Markwell & Fox, 1978), and free I^- was removed by desalting on prepacked Sephadex G-25M columns (PD-10 columns; Pharmacia) equilibrated in 0.15 M-NaCl (for C3b) or 10 mM-potassium phosphate/0.5 mM-EDTA/0.1% (w/v) Emulphogene, pH 7.0 (for CR1). Labelled CR1 (specific radioactivity 2×10^6 c.p.m./μg) was then re-bound to a column of C3u–Sepharose, equilibrated in the same buffer, and eluted with the same buffer made 0.2 M with respect to NaCl. This procedure was necessary to remove trace (contaminant) components of the detergent that had been iodinated. Autoradiography of radioiodinated material on SDS/polyacrylamide gels after electrophoresis was done with Fuji-RX X-ray film (Fuji Photo Film Co., Tokyo, Japan) with Cronex Lightning Plus intensifying screens (DuPont, Southampton, U.K.).

Proteolytic digestion of CR1

CR1 (unlabelled, or radioiodinated, as described above) was digested with trypsin, and the products of digestion were analysed by SDS/polyacrylamide-gel electrophoresis, in reducing and non-reducing conditions. Digestion products of unlabelled CR1 were assayed for cofactor activity as described above, and digestion products of radioiodinated CR1 were tested for their ability to re-bind to C3u–Sepharose as described above, under 'Radioiodination'.

RESULTS AND DISCUSSION

Isolation of CR1

In initial attempts with ion-exchange, lentil lectin and hydroxyapatite chromatography to isolate CR1 from stored plasma-reduced blood it was found that CR1 is very sensitive to proteolysis, and, although it was possible to obtain purified CR1, this material was generally partially degraded, to a form containing disulphide-linked chains of M_r about 160000+38000+25000. This form retained cofactor activity. To decrease proteolysis, it was necessary to ensure very careful removal of leucocyte material from the blood. The filtration step described (in the Materials and methods section) together with the use of proteinase inhibitors is effective in diminishing proteolysis to a negligible level.

Table 1. Localization of CR1 activity in fractions derived from human erythrocytes

The quantities of protein shown are from a preparation made with 25 units of plasma-reduced blood. For full experimental details see the text.

Fraction	Total protein (g)	Total protein (%)	Cofactor activity of CR1 (%)
Erythrocytes	1630	100	100
Supernatant after osmotic lysis	1615	99	11
Erythrocyte ghosts	12.5	1	89
Erythrocyte ghosts	12.5	100	100
Supernatant after detergent solubilization	3.4	27	96
Pellet after detergent solubilization	9.1	73	4

The yield of protein and cofactor activity in the initial stages of the preparation is shown in Table 1. Washing of the membranes of lysed erythrocytes in the Pellicon cassette system results in about 11% loss of CR1 activity into the supernatant, probably owing to formation, by shear forces, of membrane microvesicles that cross the filter membrane. Subsequent solubilization of the membrane proteins with Nonidet P40 and Triton X-100 is relatively selective, in that only 27% of the total protein is extracted, together with 96% of CR1 activity. The use of ionic detergents, e.g. cholate or deoxycholate, is impracticable here, since these detergents prevent the interaction of CR1 with ligands (C3b or C3u) and so inhibit both the assay of cofactor activity of CR1, and subsequent ligand affinity-chromatographic steps.

Ion-exchange chromatography of the soluble extract provides a high degree of purification (Fig. 1 and Table 2). CR1 activity is eluted from the gradient at approx. 0.05–0.1 M-KCl. Recovery of CR1 cofactor activity in the pool (Fig. 1) is 68–75% (Table 2). At this stage, CR1 is 5–10% pure (Fig. 2, track B) and contains multiple contaminants of M_r 25000–90000.

The assay used for CR1 will also detect the serum protein complement Factor H. Although thorough washing of the erythrocytes before lysis should remove all of the Factor H, one preparation (out of 15) did contain traces of Factor H. However, Factor H is eluted from the ion-exchange column much later (at 0.15–0.2 M-KCl) than CR1, and so any trace contamination with Factor H can be excluded at this step.

Subsequent chromatography on C3u–Sepharose (Fig. 3) results in elimination of most of the contaminants (Fig. 2, track C) with only about 10% loss of CR1 activity. A protein of M_r about 70000 also adheres to C3u–Sepharose, but is eluted earlier in the gradient than CR1. A second passage on C3u–Sepharose (not shown) is necessary to eliminate this contaminant completely (Fig. 2, track D). Other studies with this contaminant protein indicate that it binds to a wide range of Sepharose-based affinity media, and so it is unlikely to be a specific C3u-binding protein.

The final yield of pure CR1 from 25 blood donations is 3.2–5.5 mg (range observed in six preparations). The

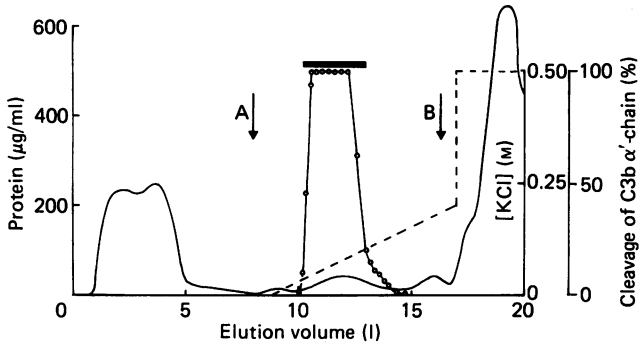

Fig. 1. Ion-exchange chromatography of the solubilized erythrocyte extract

Conditions used were as described in the Materials and methods section. Protein concentration (——) was determined by the Lowry assay. CR1 activity was detected by incubating 50 μl of column fractions with 0.4 μg of Factor I + ^{125}I-labelled C3b (10000 c.p.m.: 0.1 μg) at 37 °C for 1 h. Samples were then analysed by SDS/polyacrylamide-gel electrophoresis and autoradiography. CR1 activity is expressed as percentage cleavage of C3b α′-chain (○–○). The start of the gradient (A) and of the 0.5 M-KCl wash (B) are indicated by arrows. The pool of CR1 is indicated by a bar. Molarity of KCl is indicated by ----.

preparation shown in Table 2 had a yield of 57% of the CR1 activity compared with the detergent extract from erythrocyte membranes, or 48% compared with the activity of intact erythrocytes. Increase in the scale of the preparation up to 40 units of blood gives proportionally higher yields.

The amounts of CR1 on erythrocytes vary widely between individuals (for discussion see Fearon & Wong, 1983). Estimates of the average number of CR1 molecules per erythrocyte in pooled blood vary between about 400 and 1400 (Fearon, 1980; Iida *et al.*, 1982; Arnaout *et al.*, 1983; Walport *et al.*, 1985). The yield shown in Table 2 is consistent with a mean site number of about 500 per erythrocyte, if it is assumed that the purified protein is not partially inactivated.

Characteristics of CR1

The apparent M_r of reduced CR1 on SDS/polyacrylamide-gel electrophoresis is 240000. This corresponds to the commonest phenotype, type A (Holers *et al.*, 1984). In most preparations a faint additional band of apparent M_r 260000 is visible on Coomassie Blue-stained gels. This corresponds to phenotype B. These M_r estimates are similar to those reported by Wong *et al.* (1983). CR1 has a lower apparent M_r (204000 for type A; 220000 for type B) when analysed under non-reducing conditions. The two phenotypes are clearly visible in Fig. 4. The M_r estimates for non-reduced proteins are similar to those reported by Holers *et al.* (1984).

The amino acid and hexosamine composition of CR1 is shown in Table 3. This analysis was performed on preparations in which only the A phenotype was detectable. On the basis of typical average glycoprotein oligosaccharide structures (Wagh & Bahl, 1981; Hatton *et al.*, 1983), the glucosamine content of CR1 (about 30 residues per molecule) indicates the presence of six or seven complex *N*-linked oligosaccharides per molecule,

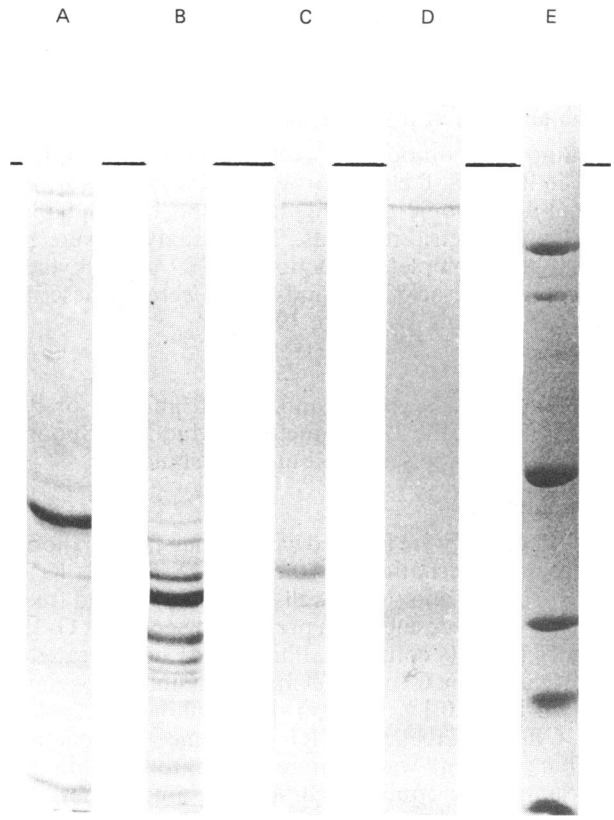

Fig. 2. SDS/polyacrylamide-gel electrophoresis of CR1 fractions

Samples were reduced and alkylated and run as described in the Materials and methods section. Gels were stained with Coomassie Blue. Track A, erythrocyte ghosts (7 μg of protein); track B, CR1 pool from ion-exchange chromatography (20 μg); track C, CR1 pool after first C3u–Sepharose step (2 μg); track D, CR1 pool after second C3u–Sepharose step (1 μg); track E, standards, myosin (M_r 205000), Factor H (apparent M_r 170000), phosphorylase *b* (M_r 92000), catalase (M_r 60000), ovalbumin (M_r 42000) and carboxypeptidase A (M_r 25000). A line marks the top of the running gel.

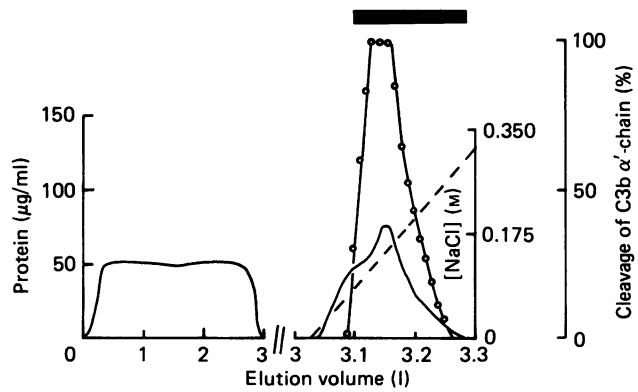

Fig. 3. Affinity chromatography of CR1 on C3u–Sepharose (first step)

Conditions were as described in the Materials and methods section. Protein concentration was determined by the Lowry assay and is shown by ——. The cofactor activity of CR1 (○–○) was detected as described in the Materials and methods section and in the legend to Fig. 1. Molarity of NaCl is shown by ----.

Table 2. Chromatographic purification of CR1

The quantities of protein shown refer to a typical preparation made with 25 units of plasma-reduced blood. For full experimental details see the text.

	Total protein (mg)	Cofactor activity of CR1 (%)
Starting material for ion-exchange chromatography	3380*	100
CR1 pool from ion-exchange chromatography	153*	73
CR1 pool from first C3u–Sepharose chromatography	8.4†	66
CR1 pool from second C3u–Sepharose chromatography	4.8†	57

* Determined by the Lowry assay.
† Determined by amino acid analysis.

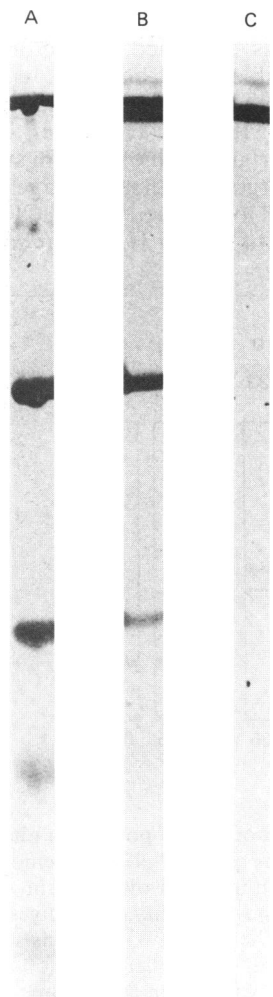

Fig. 4. SDS/polyacrylamide-gel electrophoresis of non-reduced CR1

Gels were run as described in the Materials and methods section, and were silver-stained. Track A, reduced standards, myosin (M_r 205000), phosphorylase *b* (M_r 92000), catalase (M_r 60000), ovalbumin (M_r 42000) and carboxypeptidase A (M_r 25000); track C, unreduced CR1 (150 ng), containing the A and B phenotypes; track B, a mixture of standards (as on track A) plus unreduced CR1 (as on track C). Unreduced CR 1 (A phenotype) migrates just below reduced myosin.

Table 3. Amino acid and hexosamine composition of CR1

Analyses were performed as described in the text. Cysteine was determined as *S*-carboxymethylcysteine. Serine and threonine values were extrapolated to zero hydrolysis time.

Amino acid or hexosamine	Composition (residues/100 amino acid residues)	Composition (residues/molecule)
Asp	8.39	170
Thr	4.13	84
Ser	9.97	202
Glu	11.90	241
Pro	5.36	109
Gly	11.89	241
Ala	6.53	132
Cys	2.75	56
Val	5.74	116
Met	1.33	27
Ile	3.93	80
Leu	6.90	140
Tyr	2.67	54
Phe	3.63	74
His	2.17	44
Lys	5.45	111
Arg	4.69	95
Trp	2.58	52
Total ...	100.01	2027
Glucosamine	1.51	31
Galactosamine	0.24	5

assuming that each complex oligosaccharide contains four or five *N*-acetylglucosamine residues. This would account for 6–9% of the total glycoprotein weight, and is compatible with the percentage of *N*-linked carbohydrate estimated from treatment of CR1 with endoglycosidase F (Wong *et al.*, 1983; Atkinson & Jones, 1984). Galactosamine was detected at a level of about five residues per molecule, which may indicate the presence of up to five *O*-linked sugars on the molecule. If these are of the simple disaccharide type, they represent about 0.8% of the total molecular mass of CR1. Assuming a carbohydrate content of 8% and an M_r of 240000, the CR1 polypeptide chain is about 2030 residues long (Table 3).

A characteristic feature of the functionally related molecules Factor H and C4bp is their high cysteine content (4–6%) (Gardner *et al.*, 1980; Reid & Gagnon, 1982; Sim & DiScipio, 1982). The value for CR1, however, is lower. This was confirmed by analysis after oxidation to cysteic acid. The tyrosine and tryptophan contents of CR1 are relatively high, indicating an absorption coefficient for CR1 ($A_{280}^{0.1\%}$) of 1.5 (Wetlaufer, 1962; Edelhoch, 1967).

Proteolytic digestion of CR1

Proteolytic digestion of the cofactor proteins Factor H and C4bp has provided useful information on the localization of the C3b- or C4b-binding sites on these proteins. Both proteins are relatively insensitive to trypsin in non-denaturing conditions, and digestion with trypsin results in formation of a few large well-defined fragments that are not susceptible to further proteolysis. Factor H is cleaved rapidly by trypsin into two disulphide-linked fragments of M_r about 120000–140000 and 35000. This proteolysis occurs without loss of cofactor activity for cleavage of soluble C3b by Factor I, but the trypsin-digested Factor H will no longer bind to surface-associated C3b. The binding site for soluble C3b has been shown to be located in the 35000-M_r fragment (Hong *et al.*, 1982; Sim & DiScipio, 1982; Alsenz *et al.*, 1984). Prolonged digestion of Factor H with trypsin leads to slow degradation of the 120000–140000-M_r polypeptide into two or three stable disulphide-linked fragments, without further loss of cofactor activity for cleavage of soluble C3b (Hong *et al.*, 1982; Sim & DiScipio, 1982; Alsenz *et al.*, 1984). Similarly the 75000-M_r monomer polypeptide of C4bp is rapidly cleaved by trypsin to form two stable disulphide-linked fragments of M_r 36000 and 38000. This occurs without loss of cofactor activity for cleavage of soluble C4b (Reid & Gagnon, 1982). Chymotryptic cleavage of C4bp forms two stable non-disulphide linked chains of M_r 25000 and 48000. Cofactor activity for soluble C4b cleavage is located in the 48000-M_r fragment (Nagasawa *et al.*, 1982).

The proteolytic digestion of CR1 was investigated for comparison with Factor H and C4bp, and to determine whether large stable fragments suitable for structural investigation could be obtained. The fragmentation pattern, as determined by SDS/polyacrylamide-gel electrophoresis, and the activities of the fragmented material are shown in Fig. 5.

CR1 is degraded rapidly by trypsin to a form containing two disulphide-linked chains of about M_r 160000 and 65000 (Fig. 5, 1 min). The 65000-M_r chain is further degraded to disulphide-linked fragments of M_r 38000 and 25000. These remain disulphide-linked to the 160000-M_r fragment. Further incubation (5–15 min) results in cleavage of the 38000-M_r and 25000-M_r chains to products of M_r less than 25000, and concomitant cleavage of the 160000-M_r chain to 140000-M_r and then to 90000-M_r fragments. These fragments are not disulphide-linked to any other large fragments, and no other intermediates of M_r greater than 25000 were identified. Digestion from 25 to 60 min results in breakdown of the 90000-M_r fragment to a fragment of M_r 70000, which is then cleaved to disulphide-linked fragments of M_r 40000 and 25000. These products are also unstable and are degraded slowly to fragments of M_r less than 25000.

CR1 appears to lose the capacity to bind to solid-phase C3u after a single cleavage by trypsin. At the point where no intact CR1 is visible (Fig. 5, 2 min), binding to C3u–Sepharose is abolished. Cofactor activity for the cleavage of soluble C3b, however, is retained during digestion, although there is a loss of about 70% of activity during the first 10 min. Thereafter cofactor activity (25–30% of the original value) remains stable even though the protein undergoes further cleavage.

The pattern of loss of activity of CR1 on proteolysis is similar to that of Factor H, in that cofactor activity is

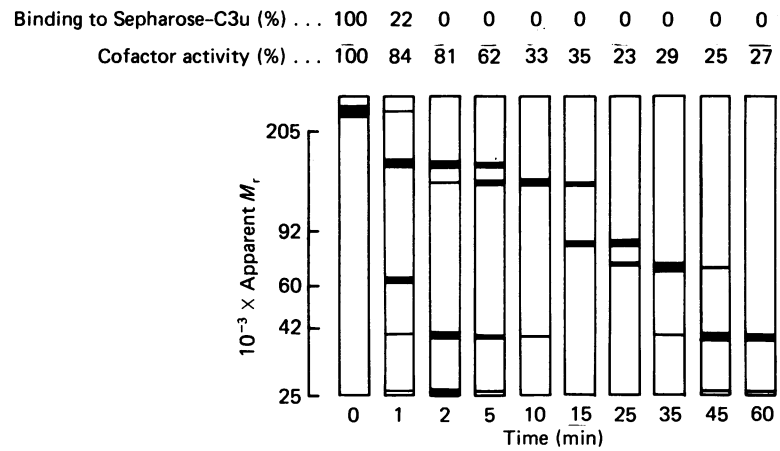

Fig. 5. Trypsin digestion of CR1

Radioiodinated CR1 (2 μg; 4×10^6 c.p.m.) was digested with trypsin (40 ng) in 400 μl of 10 mM-potassium phosphate/0.5 mM-EDTA/0.1% (w/v) Emulphogene, pH 7.0, for various times at 37 °C. Three portions (each 10 μl) were removed at each time point. Reaction was stopped by addition of a 2-fold molar excess of soya-bean trypsin inhibitor over trypsin. One portion was diluted with 240 μl of the same buffer containing bovine serum albumin (1 mg/ml), and the ability of the digested CR1 to bind to C3u–Sepharose was tested as described in the Materials and methods section. The other two portions were analysed by SDS/polyacrylamide-gel electrophoresis under reducing and non-reducing conditions. Unlabelled CR1 (50 μg) was digested under the same conditions with 1 μg of trypsin in 10 ml total volume. Portions were withdrawn at the same time intervals to measure cofactor activity for cleavage of soluble C3b and for analysis by SDS/polyacrylamide-gel electrophoresis, as described in the Materials and methods section. A diagram of the pattern obtained on SDS/polyacrylamide-gel electrophoresis of reduced material is shown. This pattern is a summary of results from autoradiography at different exposure times and from Coomassie Blue staining, since some bands (particularly the 25000-M_r and 38000-M_r bands shown at 1–10 min) were poorly radiolabelled. M_r values of standards (as in Fig. 4) are shown. Disulphide linkage of the products is described in the text.

retained, whereas the ability to interact with solid-phase C3u (or C3b) is rapidly lost. This indicates that, as with Factor H (Hong *et al.*, 1982; Sim *et al.*, 1983; Alsenz *et al.*, 1984), interaction of CR1 with soluble C3b differs from the interaction with surface-bound C3u or C3b.

The general pattern of trypsin degradation of CR1 is not similar to those of Factor H or C4bp. For CR1, degradation is rapid and continuous, with multiple intermediates. No stable products of 25000 M_r greater than are formed, and so trypsin digestion in non-denaturing conditions does not generate large fragments suitable for structural studies.

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