Biochem. J. (1979) **177**, 531–540
Printed in Great Britain

Purification and Characterization of Subcomponent C1q of the first Component of Bovine Complement

By R. DUNCAN CAMPBELL, NUALA A. BOOTH and JOHN E. FOTHERGILL
Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

(*Received* 30 *May* 1978)

Bovine C1q, a subcomponent of the first component of complement, was purified in high yield by a combination of euglobulin precipitation, and ion-exchange and molecular-sieve chromatography on CM-cellulose and Ultrogel AcA 34. Approx. 12–16mg can be isolated from 1 litre of serum, representing a yield of 13–18%. The molecular weight of undissociated subcomponent C1q, as determined by equilibrium sedimentation, is 430000. On sodium dodecyl sulphate/polyacrylamide gels under non-reducing conditions, subcomponent C1q was shown to consist of two subunits of mol.wts. 69000 and 62000 in a molar ratio of 2:1. On reduction, the 69000-mol.wt. subunit gave chains of mol.wts. 30000 and 25000 in equimolar ratio, and the 62000-mol.wt. subunit decreased to 25000. The amino acid composition, with a high value for glycine, and the presence of hydroxyproline and hydroxylysine, suggests that there is a region of collagen-like sequence in the molecule. This is supported by the loss of haemolytic activity and the degradation of the polypeptide chains of subcomponent C1q when digested by collagenase. All of these molecular characteristics support the structure of six subunits, each containing three different polypeptide chains, with globular heads connected by collagen triple helices as proposed by Reid & Porter (1976) (*Biochem. J.* **155**, 19–23) for human subcomponent C1q. Subcomponent C1q contains approx. 9% carbohydrate; analysis of the degree of substitution of the hydroxylysine residues revealed that 91% are modified by the addition of the disaccharide unit Gal-Glc. Bovine subcomponent C1q generates full C1 haemolytic activity when assayed with human subcomponents C1r and C1s.

Complement is a complex mixture of plasma proteins that play an important part in defence against infection. Activation of the system can take place by two main routes, the classical and alternative pathways, both leading to activation of the membrane attack system, which can result in cytolysis (reviewed by Fothergill & Anderson, 1978). The classical pathway is activated mainly by antigen–antibody aggregates, which interact with the first component of complement, C1, a Ca^{2+}-dependent complex of three proteins, C1q, C1r and C1s (Lepow *et al.*, 1963). The binding of the first component of complement to the Fc portion of immunoglobulin takes place through the C1q subcomponent (Yasmeen *et al.*, 1976). The binding of subcomponent C1q causes activation of subcomponent C1r in the C1 complex, by the splitting of probably a single peptide bond (Gigli *et al.*, 1976; Sim *et al.*, 1977), which in turn allows activation of subcomponent C1s proenzyme (Ziccardi & Cooper, 1976; Sim *et al.*, 1977) to yield the fully active C1 complex.

Abbreviations used: SDS, sodium dodecyl sulphate; dansyl, 5-dimethylaminonaphthalene-1-sulphonyl; the symbols for complement components conform to the recommendations of the World Health Organization (1968) [see also *Immunochemistry* (1970)].

Human subcomponent C1q (Yonemasu & Stroud, 1971; Calcott & Muller-Eberhard, 1972; Reid *et al.*, 1972) and rabbit subcomponent C1q (Reid *et al.*, 1972; Volanakis & Stroud, 1972; Lowe & Reid, 1974) have both been well characterized. Each has been shown to have an unusual amino acid composition, consistent with there being a region of collagen-like amino acid sequence in the molecule, and this has now been confirmed by sequence studies on the three polypeptide chains of human subcomponent C1q (Reid, 1974, 1976, 1977).

Detailed structural studies on human complement components have been hampered by the poor availability of blood. Similar disadvantages apply to small animals such as the rabbit and the guinea pig. Although very little characterization has been carried out on the complement system in larger animals, it seemed worthwhile to investigate their potential as a source of complement components. The ready availability of bovine blood made this the

system of choice, as it has also been for detailed study of many coagulation factors. Comparison of the structures of human and bovine components may help to elucidate those features which are important for biological activity.

The present paper describes the purification of bovine subcomponent C1q in high yield by rapid and simple chromatographic procedures. Characterization has also been carried out, and comparison of subcomponent C1q with human and rabbit subcomponent C1q suggests that the bovine subcomponent is very similar to the others. Together with the bovine C1r and C1s subcomponents (R. D. Campbell, N. A. Booth & J. E. Fothergill, unpublished work), which are also analogous to their human counterparts, bovine C1q will provide adequate material for a detailed study of the C1 complex, and of its interaction with bovine C4 (Booth *et al.*, 1979).

Materials and Methods

Materials

The sources of reagents used were as follows: sheep erythrocytes in Alsevers solution, Tissue Culture Services, Slough, Berks., U.K.; guinea-pig serum, Wellcome Reagents, Beckenham, Kent, U.K.; benzamidine hydrochloride hydrate, Aldrich Chemical Co., Gillingham, Dorset, U.K.; CM-cellulose 52, Whatman, Maidstone, Kent, U.K.; DEAE-Sephadex A-50, Pharmacia (Great Britain) Ltd., London W.5, U.K.; Ultrogel AcA 34, LKB Instruments Ltd., South Croydon, Surrey, U.K.; agarose, SDS, polyacrylamide, *NN'*-methylenebisacrylamide, BDH, Poole, Dorset, U.K.; sialic acid, Coomassie Brilliant Blue R (C.I. no. 42660), collagenase type 111 (chromatographically purified), Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K.; rabbit anti-(human subcomponent C1q), Hoechst Pharmaceuticals, Hounslow, Middx., U.K.

Methods

Isolation of subcomponent C1q. Bovine blood was collected at the slaughterhouse in 1-litre plastic centrifuge bottles and allowed to clot at 37°C for 30min and used immediately. All subsequent manipulations were performed at 4°C unless otherwise stated. Measurements of pH and conductivity were made at room temperature.

Serum was separated from the clot by centrifugation at 1800g for 1h. The euglobulin fraction of 5 litres of serum was prepared by dialysing the serum against three changes of 40 litres of 8.5mM-NaCl/13.3mM-sodium phosphate buffer (pH6.0, conductivity 1.8mmho/cm) strictly at 0°C for 36h. The euglobulin fraction thus formed was centrifuged at 1800g for 1h, washed with dialysis buffer, and resuspended by gentle hand homogenization in 500ml of 10mM-EDTA/100mM-NaCl/10mM-benzamidine/2.5mM-iodoacetamide/0.2mM-1,10-phenanthroline/25mM-potassium phosphate buffer (pH 7.4, conductivity 16.0mmho/cm).

The euglobulin sample was clarified by centrifugation at 20000g for 30min and applied to a column (5cm×30cm) of DEAE-Sephadex A-50 equilibrated with the same buffer. The column was eluted at a rate of 75ml/h. Subcomponent C1q was eluted with the starting buffer, and the fractions were pooled and dialysed against 100mM-NaCl/10mM-EDTA/200mM-sodium acetate buffer (pH5.3, conductivity 21mmho/cm). The solution (950ml) was chromatographed on a column (5cm×36cm) of CM-cellulose equilibrated with the NaCl/EDTA/acetate buffer, pH5.3, at a flow rate of 100ml/h. After the first protein peak had been eluted, a linear gradient composed of 2500ml of 100mM-NaCl/10mM-EDTA/200mM-sodium acetate buffer, pH5.3, and 2500ml of 400mM-NaCl/10mM-EDTA/200mM-sodium acetate buffer, pH5.3, was begun. The tubes containing the subcomponent C1q haemolytic activity were pooled, concentrated by ultrafiltration to 18ml and dialysed against 100mM-NaCl/10mM-EDTA/200mM-sodium acetate, pH5.3. This solution was applied to a column (2.5cm×140cm) of Ultrogel AcA 34 equilibrated with the same buffer and eluted by upward flow at a rate of 15ml/h. The tubes containing the subcomponent C1q haemolytic activity were pooled and concentrated, and represented the finally purified subcomponent C1q.

Haemolytic assay of subcomponent C1q activity. Veronal-buffered saline, I 0.065, pH7.3, containing 1mM-$MgCl_2$ and 0.15mM-$CaCl_2$ (VBS buffer) and veronal-buffered saline, I 0.065, pH7.3, containing 10mM-EDTA (VBS-EDTA buffer) were prepared by the method of Rapp & Borsos (1970). EAC4 cells were prepared from sheep erythrocytes, rabbit antiserum and guinea-pig serum as described by Mayer (1961). Functionally pure guinea-pig component C2 was prepared as described by Nelson *et al.* (1966). A mixture of functionally pure bovine subcomponents C1r and C1s was prepared by ion-exchange chromatography of euglobulin on DEAE-Sephadex A-50 at pH7.4 (R. D. Campbell, N. A. Booth & J. E. Fothergill, unpublished work). The mixture of subcomponents C1r and C1s was stored at −70°C in 0.2ml samples. The late components of complement (C3–C9) were supplied in the form of whole guinea-pig serum diluted 1 to 25 with VBS buffer/EDTA.

To assay for subcomponent C1q haemolytic activity, 0.1ml serial dilutions of the solution to be tested were made in VBS buffer; 0.2ml of the C1r/C1s mixture in VBS buffer was added along with 0.1ml of EAC4 cells (1.5×10^8/ml). After incubation at 37°C for 15min, 0.1ml of component C2 in VBS buffer was added and the cells were incubated at

37°C for 10min. Then 0.5ml of whole guinea-pig serum, diluted 1:25 with VBS buffer/EDTA, was added and the mixture was incubated at 37°C for 30min. The cells were centrifuged at 1500*g* for 2min and the A_{412}^{1cm} values of the supernatants read. All appropriate controls were included in each assay. Results were expressed in CH_{50} units, i.e. the reciprocal of the dilution of test material causing 50% lysis of the EAC4 cells.

Protein determination. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Quantitative amino acid analysis. Amino acid analyses were performed by the method of Moore & Stein (1963) in a Locarte amino acid analyser. Samples were hydrolysed in duplicate in 5.7M-HCl at 110°C for 24, 48 and 72h in evacuated tubes. Destruction of the labile amino acids was corrected for by extrapolation to zero hydrolysis time. Valine, leucine and isoleucine values are those determined for the 72h hydrolysis. Cysteine was determined as cysteic acid after performic acid oxidation as described by Hirs (1967). Tryptophan was determined spectrophotometrically by the method of Beavan & Holiday (1952).

Carbohydrate analysis. Total hexose was determined by the orcinol/H_2SO_4 procedure of Winzler (1955) as modified by François *et al.* (1962). Sialic acid was determined by the method of Aminoff (1961) after hydrolysis of the subcomponent C1q in 0.2M-H_2SO_4 at 85°C for 1h. Hexosamine was determined with the amino acid analyser by the method of Spiro (1972) on samples previously hydrolysed in 4M-HCl for 4h at 110°C.

To determine the degree of substitution of carbohydrate on hydroxylysine residues, samples (0.096 mg) of subcomponent C1q were subjected to hydrolysis in 2M-NaOH in polypropylene bottles at 110°C for 16h. After desalting, glycosides were determined directly on a Locarte analyser (Isemura *et al.*, 1976). Samples were quantified by using Hyl-Gal-Glc and Hyl-Gal prepared from bovine bone collagen.

Polyacrylamide-gel electrophoresis in the presence of SDS. Polyacrylamide-gel electrophoresis in the presence of SDS was performed as described by Weber *et al.* (1972). Samples were run on 7.5% polyacrylamide gels in 0.1% SDS/0.1M-sodium phosphate buffer, pH7.2. Samples were prepared by incubation at 100°C for 2min in either 1% SDS/1% mercaptoethanol/0.01M-sodium phosphate buffer, pH7.2, or 1% SDS/4M-urea/0.01M-sodium phosphate buffer, pH7.2. The gels were stained for protein with 0.25% Coomassie Brilliant Blue in methanol/acetic acid/water (5:1:5, by vol.) and destained in 7% acetic acid/9.5% methanol solution. Carbohydrate was stained by the method of Kapitany & Zebrowski (1973). Molecular weights were obtained by interpolation from a linear semi-logarithmic plot of apparent molecular weight against migration distance by using five standard proteins: bovine immunoglobulin G, bovine serum albumin, ovalbumin, lactate dehydrogenase and β-lactoglobulin. The gels were scanned for protein and carbohydrate stain in a Unicam SP.1800 spectrophotometer with the Unicam SP.1809 scanning-densitometer accessory.

Analytical ultracentrifugation. Sedimentation-velocity analysis was carried out in a Beckman model E ultracentrifuge with schlieren optics. The sedimentation coefficient of subcomponent C1q (in 430mM-sodium acetate buffer, pH5.2) was determined at 20°C by using a single-sector cell and a rotor speed of 59780rev./min.

The molecular weight of subcomponent C1q (1.5 mg/ml in 100mM-NaCl/10mM-EDTA/25mM-potassium phosphate buffer, pH7.4) was determined by sedimentation equilibrium as described by Lamm (1929). A rotor speed of 5784rev./min was used and photographs were taken by using the schlieren optical system. The partial specific volume was calculated from the amino acid (Schachman, 1957) and carbohydrate compositions (Gibbons, 1966).

Preparation of antiserum. Antiserum to subcomponent C1q was produced in rabbits by injection of 200μg of purified protein in complete Freund's adjuvant into the region of the popliteal lymph nodes, and intramuscularly into two sites in the back. After 3 weeks the rabbits were given an intramuscular and intravenous booster injection of 200μg of subcomponent C1q in complete Freund's adjuvant. After a further 10 days the rabbits were bled, and thereafter at 10-day intervals.

Immunodiffusion, immunoelectrophoresis and radial immunodiffusion

For immunodiffusion, the method of Ouchterlony (1958) was followed. Plates were prepared by using 0.6% agarose in 150mM-NaCl/50mM-Tris/50mM-glycine buffer, pH8.6 containing 0.2% NaN_3.

Immunoelectrophoresis was performed by a modification of the method of Scheidegger (1955) using 1% agarose in 25mM-Tris/HCl/25mM-glycine/75mM-NaCl/10mM-EDTA, pH8.6. Electrophoresis was performed for 2h at 4°C with a potential gradient of 6V/cm.

Radial immunodiffusion was carried out by a modification of the method of Mancini *et al.* (1965). Plates were prepared with 1% agarose containing 0.3–0.5% (v/v) antiserum to C1q in the buffered saline used for Ouchterlony analysis. The plates were standardized by using a purified preparation of subcomponent C1q, the concentration of which was determined by amino acid analysis.

N-Terminal analysis. Qualitative *N*-terminal analysis was carried out by the dansyl method described by Weiner *et al.* (1972).

Enzymic digestion of subcomponent C1q. Sub-

component C1q (0.5mg in 0.5ml 0.1M-Tris/HCl buffer, pH7.4, containing 0.01M-$CaCl_2$) was incubated with 25μg of collagenase (Sigma type 111) at 37°C. Samples (50μl) were taken from the digest and control tubes at various time intervals from 0 to 24h and frozen immediately at −70°C. Haemolytic activity and mobilities on SDS/polyacrylamide gels were determined immediately after thawing.

Results

Isolation of subcomponent C1q

The recovery of subcomponent C1q in the euglobulin, which represents approx. 8% of the protein in serum, ranged from 65 to 75%. No subcomponent C1q was detected in the pseudoglobulin. The material not retained on the DEAE-Sephadex column, representing 48% of the applied protein, contained all the subcomponent C1q haemolytic activity. The recovery of haemolytic activity was 73% of that applied to the column (Table 1). Ion-exchange chromatography on CM-cellulose (Fig. 1) resulted in 99% of the applied protein being eluted with the starting buffer. Subcomponent C1q haemolytic activity was retained and later eluted by an NaCl gradient in the conductivity range 23–27mmho/cm. Final purification was achieved by molecular-sieve chromatography on a column of Ultrogel AcA 34 (Fig. 2). Approx. 58% of the protein applied to the column was eluted near the void volume of the column. The recovery of haemolytic activity at this step was 76% (Table 1).

The yield of bovine subcomponent C1q from serum was approx. 12–16mg/litre. This represents a yield, as measured by radial immunodiffusion, of 13–18% of the starting material. The specific haemolytic activity of the purified subcomponent C1q was reproducibly in the range 1.5×10^4–2.5×10^4 C1q H_{50} units/mg.

Serum concentration of subcomponent C1q

The serum concentration of subcomponent C1q, as measured by radial immunodiffusion, was reproducibly in the range 85–95μg/ml. Samples of serum

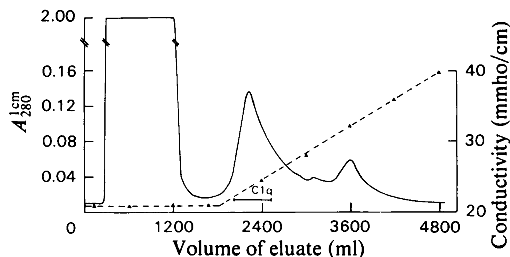

Fig. 1. *Chromatography of subcomponent C1q on CM-cellulose*

The subcomponent C1q fraction (950ml) from the DEAE-Sephadex column was loaded on to a column (5cm×36cm) of CM-cellulose equilibrated in 100mM-NaCl/10mM-EDTA/200mM-sodium acetate buffer, pH5.3. After the first protein peak had been eluted, a linear NaCl gradient (as described in the Materials and Methods section) was applied. ——, A_{280}; ▲, conductivity (mmho/cm).

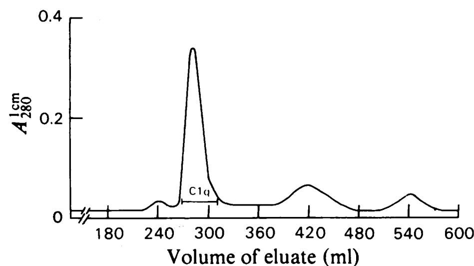

Fig. 2. *Chromatography of subcomponent C1q on Ultrogel AcA* 34

The subcomponent C1q fraction (512ml) from the CM-cellulose column was concentrated to 18ml, dialysed against 100mM-NaCl/10mM-EDTA/200mM-sodium acetate buffer, pH5.3, and loaded on to a column (2.5cm×140cm) of Ultrogel AcA 34 equilibrated in the same buffer. ——, A_{280}.

Table 1. *Purification of subcomponent C1q from 5 litres of serum*

For details of purification see the text. ND indicates that activity of subcomponent C1q in serum was not measured as results were not reproducible.

Purification step	Total protein (mg)	$10^{-6}\times$Total CH_{50} units	Specific activity (CH_{50} units/mg)	C1q* (mg)	Yield (%)
Serum	289000	ND	ND	450	100
Euglobulin	24167	5.02	208	311	69
DEAE-Sephadex	11667	3.68	315	210	47
CM-cellulose	137.5	2.14	15564	115	26
Ultrogel	79	1.63	20633	70	15.6

* Determined by radial immunodiffusion.

taken from the jugular veins of bulls gave a concentration of subcomponent C1q in the range 100–110 μg/ml. This indicates that the serum collected routinely from the throat was only slightly diluted.

Sedimentation velocity

Subcomponent C1q in 430mM-sodium acetate buffer, pH 5.2, yielded a single symmetrical peak in the analytical ultracentrifuge. The $s_{20,w}$, measured at a protein concentration of 3.35 mg/ml, was calculated to be 9.74 S.

Effect of heat treatment on bovine subcomponent C1q

Over 90% of bovine subcomponent C1q was precipitated on heat treatment at 56°C for 30 min, which caused complete loss of the haemolytic activity and of the ability to form precipitin lines in immunodiffusion.

Immunodiffusion

Purified subcomponent C1q gave a single precipitin line in Ouchterlony double-diffusion in agarose gels against rabbit antiserum raised against the same material. A single precipitin arc in the γ-globulin region was obtained when purified subcomponent C1q was subjected to immunoelectrophoresis and tested against anti-(bovine subcomponent C1q). Bovine subcomponent C1q gave a precipitin line in Ouchterlony double-diffusion when tested against rabbit anti-(human subcomponent C1q), but human subcomponent C1q formed a spur with this line. No precipitin line was detectable when human C1q subcomponent was tested against anti-(bovine subcomponent C1q) at comparable concentrations.

Reconstitution of subcomponent C1 haemolytic activity with a mixture of bovine and human subcomponents

With molar proportions of 1:4:4 for subcomponents C1q, C1r and C1s respectively, which has been shown to be optimal for human component C1 activity (Gigli *et al.*, 1976), we find that bovine subcomponent C1q, when mixed with human subcomponents C1r and C1s, generates almost complete component C1 haemolytic activity. When bovine subcomponent C1q and human subcomponents C1q, C1r and C1s were assayed separately on the EAC4 cells, no significant component C1 haemolytic activity could be detected (Table 2). Similarly no activity was detected in mixtures of bovine subcomponent C1q with either human subcomponent C1r or subcomponent C1s.

Absorption coefficient of subcomponent C1q

The $A^{1\%}_{1cm}$ of subcomponent C1q in 0.5M-acetic acid, after drying to constant weight, was 7.20 at 280 nm.

Table 2. *Reconstitution of component C1 haemolytic activity with a mixture of bovine and human subcomponents*

The molar proportions of the subcomponents (C1q/C1r/C1s) were 1:4:4. Activity is expressed as a percentage of human subcomponents C1q, C1r and C1s assayed together. Abbreviations used: bo, bovine; hu, human.

Subcomponent present	Component C1 haemolytic activity (H_{50} units/ml)	(%)
$C1q^{bo}$, $C1q^{hu}$, $C1r^{hu}$, or $C1s^{hu}$ alone	1500	3
$C1q^{bo}+C1r^{hu}$	1500	3
$C1q^{bo}+C1s^{hu}$	1500	3
$C1r^{hu}+C1s^{hu}$	1500	3
$C1q^{bo}+C1r^{hu}+C1s^{hu}$	40000	95
$C1q^{hu}+C1r^{hu}+C1s^{hu}$	42000	100

Table 3. *Amino acid compositions of bovine and human subcomponents C1q*

Samples of subcomponent C1q (60 μg) were hydrolysed for 24, 48 and 72 h in duplicate. Experimental details are given in the text.

Amino acid	Amino acid composition (residues/100 residues): Bovine subcomponent C1q	Human subcomponent C1q (Reid *et al.*, 1972)
Asp	8.93	8.19
Thr	5.80	5.62
Ser	6.88	4.72
Glu	9.54	8.39
Pro	5.71	7.05
Gly	16.00	17.09
Ala	4.80	4.36
Cys	1.97	1.73
Val	5.60	5.95
Met	1.47	1.55
Ile	2.40	4.26
Leu	4.89	6.20
Tyr	3.01	3.57
Phe	5.02	4.23
His	2.10	1.41
Lys	4.30	4.04
Arg	4.71	4.55
Hyp	4.54	4.51
Hyl	1.75	2.09
Trp	0.58	0.50

Amino acid and carbohydrate composition of subcomponent C1q

The amino acid composition of subcomponent C1q is given in Table 3. A notable feature of the analysis is the high glycine value and the presence of hydroxyproline and hydroxylysine. The overall

Table 4. *Carbohydrate compositions of bovine and human subcomponents C1q*

Full experimental details are given in the text. Abbreviation used: ND, not determined.

	Carbohydrate composition (g/100g of protein)	
Sugar	Bovine subcomponent C1q	Human subcomponent C1q (Reid *et al.*, 1972)
Hexose	8.30	6.95
Glucosamine	0.53	0.59
Galactosamine	0.08	0.06
Fucose	ND	0.11
Sialic acid	0.20	0.32
Total	9.11	8.03

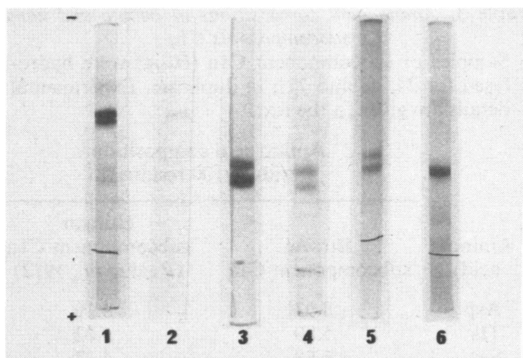

Fig. 3. *SDS/polyacrylamide-gel electrophoresis of subcomponent C1q*

1 and 2, Unreduced subcomponent C1q stained for protein and carbohydrate respectively; 3 and 4, reduced subcomponent C1q stained for protein and carbohydrate respectively; 5, 69000-mol.wt. subunit eluted and electrophoresed under reducing conditions; 6, 62000-mol.wt. subunit eluted and electrophoresed under reducing conditions.

composition is very similar to that of human and rabbit subcomponents C1q (Reid *et al.*, 1972). The carbohydrate composition of subcomponent C1q is given in Table 4. Most of it is in the form of neutral hexoses and is slightly higher than the corresponding carbohydrate compositions of human and rabbit subcomponents C1q (Reid *et al.*, 1972); 87% of the hexosamines in bovine subcomponent C1q is glucosamine.

Analyses of the carbohydrate unit attached to hydroxylysine revealed that 91% of the hydroxylysine residues present in subcomponent C1q are substituted with the disaccharide unit galactosylglucose. This represents about half of the neutral hexose content. No hydroxylysylgalactose was detected.

N-Terminal analyses

Several *N*-terminal analyses of subcomponent C1q by the dansyl method produced only dansylglutamic acid.

Molecular weights of subcomponent C1q and its polypeptide chains under reducing and non-reducing conditions

The molecular weight determined by sedimentation equilibrium under non-dissociating conditions was 430000±15000. With a calibrated Ultrogel AcA 34 column, subcomponent C1q was eluted in the mol.wt. range 340000–360000. The apparent molecular weights of the unreduced subunits of bovine subcomponent C1q on polyacrylamide gels in the presence of SDS (Fig. 3) were found to be 69000 and 62000 with the use of unreduced marker proteins. The molar ratio of the unreduced subunits was always found to be 2:1 respectively. Most of the carbohydrate in subcomponent C1q appears to reside in the 69000-mol.wt. subunit, with very little in the 62000-mol. wt. subunit. Reduction of subcomponent C1q gave two bands of apparent mol. wts. 30000 and 25000. The molar ratio of the reduced subunits was 1:2. The proportions of the carbohydrate stain in

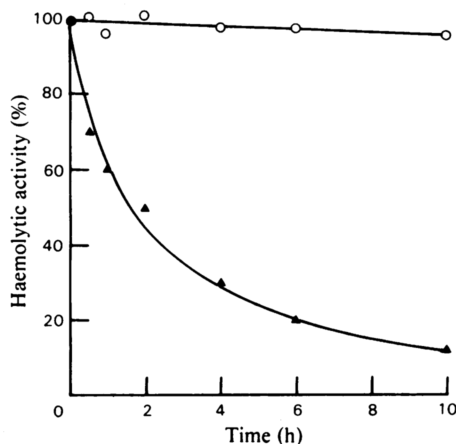

Fig. 4. *Collagenase digestion of subcomponent C1q*

Subcomponent C1q (0.5mg) was digested with collagenase (25μg) as described in the Materials and Methods section. Samples were removed at various time intervals from 0 to 24h and frozen immediately at −70°C until required for haemolytic activity determination. The haemolytic activity expressed as a percentage of the initial activity is plotted against time of digestion. ▲, Collagenase digestion; ○, control without collagenase.

each band was 1.6 to 1.0, taking the 25000-mol. wt. band as 1.0.

To determine from which unreduced subunit the reduced bands are derived, unreduced subcomponent C1q was run on 7.5% polyacrylamide gels in the presence of SDS. The appropriate areas of the gels corresponding to the unreduced bands were excised and protein was eluted from the gel slices by adding a small portion of 4M-urea in 0.01M-sodium phosphate buffer, pH7.2, and incubating at room temperature overnight. The bands were then run on 7.5% polyacrylamide gels in the presence of SDS under reducing conditions (Fig. 3). One subunit with an apparent mol.wt. of 30000 and one with an apparent mol.wt. of 25000 are derived from the 69000-mol.wt. unreduced subunit. The molar ratio of these bands was 1.1:1.0. The other subunit of apparent mol.wt. 25000 is derived from the 62000-mol.wt. unreduced subunit.

Effect of ionic strength on the behaviour of the unreduced subunits of subcomponent C1q on polyacrylamide gels in the presence of SDS

The behaviour of the unreduced subunits of subcomponent C1q on 7.5% polyacrylamide gels in the presence of SDS appears not to be influenced by high salt concentrations. Even when loaded on to the gel in 0.5M-NaCl, no higher-molecular-weight material could be detected on the gels and the molar ratio of the bands was consistently 2:1.

Collagenase digestion of native bovine subcomponent C1q

Digestion of subcomponent C1q with collagenase at 37°C caused a rapid decrease in haemolytic activity compared with the control sample held at 37°C (Fig. 4). Analyses of the urea-dissociated digest on 7.5% polyacrylamide gels in the presence of SDS showed four degradation products (Fig. 5). Scanning of the gels revealed that, after 6h of incubation with collagenase, 70% of the 62000-mol.wt. unreduced subunit of subcomponent C1q had been completely digested, whereas only 30% of the 69000-mol.wt. unreduced subunit had been digested. After 24h of incubation both unreduced subunits of subcomponent C1q had been completely digested by collagenase. Incubation of hen ovalbumin with collagenase under the same conditions gave no degradation products, thereby showing that the collagenase was not contaminated with other proteolytic enzymes.

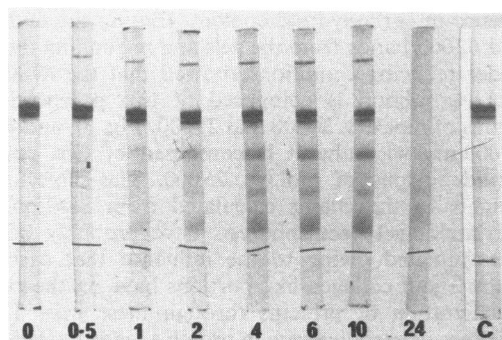

Fig. 5. *SDS/polyacrylamide-gel electrophoresis of subcomponent C1q digested with collagenase*
Numbers refer to times (h) at which samples were removed. C refers to control sample held at 37°C for 24h.

Discussion

The isolation of haemolytically active bovine subcomponent C1q in relatively high yield has been possible by using simple chromatographic techniques. Starting with 5 litres of bovine serum it has been possible to isolate 60–80mg of subcomponent C1q (Table 1). The euglobulin precipitate was also used as the starting material for the purification of bovine subcomponents C1r and C1s (R. D. Campbell, N. A. Booth & J. E. Fothergill, unpublished work). To prevent proteolytic activation of these subcomponents, serum was dialysed strictly at 0°C. This has been shown by Ziccardi & Cooper (1976) to prevent proteolytic activation of the corresponding human subcomponents. The stability of unactivated component C1 in whole serum is probably due to proteinase inhibitors, which are present in large excess. However, in separation of the euglobulin material by dialysis, a large proportion of the proteinase inhibitors in serum remains in the pseudoglobulin (Davie *et al.*, 1976). To prevent proteolytic activation of subcomponents C1r and C1s by any contaminating proteinases, it was found necessary to redissolve the euglobulin precipitate in a buffer containing 10mM-benzamidine. Benzamidine has been used extensively in the isolation of bovine Factor VIII (Schmer *et al.*, 1972), Factor IX, Factor X (Fujikawa *et al.*, 1972, 1973) and Factor VII (Kisiel & Davie, 1975) and has been shown to prevent proteolytic degradation of these proteins. Iodoacetamide, to inactivate thiol-dependent enzymes, and 1,10-phenanthroline, to inactivate the zinc-dependent tissue collagenases, were also contained in the buffer used to dissolve the euglobulin.

Previous studies on the bovine complement system indicated that bovine serum was non-haemolytic in the standard assay system with sensitized sheep erythrocytes. However, in assays for the early complement components with reagents prepared from guinea-pig serum, it was shown that bovine serum contained component C1 activity (Rice & Crowson, 1950). Since then, bovine serum has been shown to

react with sensitized sheep erythrocytes to form an intermediate that can be lysed by EDTA-treated guinea-pig serum and that this intermediate is EAC142 (Fong *et al.*, 1971*a,b*). This, together with work by Barta & Barta (1972), who showed that whole bovine complement can be assayed best with guinea-pig or rabbit erythrocytes, but not with sheep erythrocytes, suggests that incompatibility between the late-acting components of bovine complement and sheep erythrocytes was responsible for previous reports that bovine serum was non-haemolytic. Our assay system is based on the interaction of bovine C1 components with guinea-pig complement components on sensitized sheep erythrocytes. Bovine component C1 interacts with EAC4 cells, forming a cell-bound complex that is stable to the usual washing procedures and that is capable of cleaving guinea-pig component C2, thus causing activation of the membrane-attack system and lysis of the cells.

The concentration of bovine subcomponent C1q in serum collected at the slaughterhouse is reproducibly in the range 85–95 μg/ml. To check that the serum had not been substantially diluted by other body fluids, the serum concentration of subcomponent C1q was measured in samples of venous blood taken from live bulls. Since the concentration of subcomponent C1q in these samples is in the range 100–110 μg/ml, dilution of the serum collected at the slaughterhouse is very slight.

The amino acid and carbohydrate compositions of bovine subcomponent C1q (Tables 3 and 4) are similar to those reported for the human and rabbit subcomponents (Reid *et al.*, 1972; Yonemasu *et al.*, 1971; Calcott & Muller-Eberhard, 1972). Notable features of the amino acid composition are the high glycine value and the presence of hydroxyproline and hydroxylysine, suggestive of a collagen-like sequence in subcomponent C1q. This was supported by the susceptibility of the haemolytic activity of subcomponent C1q to digestion by purified collagenase (Fig. 4). A collagen-like amino acid sequence of about 78 residues has been found in each of the three chains of human subcomponent C1q (Reid, 1974, 1976, 1977). This region of the C1q molecule is common to the human, rabbit and bovine molecules and appears to be essential for the biological activity of subcomponent C1q.

Analyses of the carbohydrate attached to subcomponent C1q revealed the presence of the disaccharide unit galactosylglucose linked to hydroxylysine. No hydroxylysylgalactose could be detected, and approx. 91% of the hydroxylysine residues were modified by addition of the disaccharide unit. The functional significance of this carbohydrate moiety is not known, but it can be compared with type-IV collagen, in which approx. 95% of the hydroxylysine residues with carbohydrate attached are substituted with the disaccharide unit (Kefalides, 1973). It has been shown for rabbit subcomponent C1q (Yonemasu *et al.*, 1971) and human subcomponent C1q (Calcott & Muller-Eberhard, 1972) that some of the hydroxylysine residues are substituted. However, the nature and degree of substitution are not known. The small amounts of glucosamine and sialic acid detected in subcomponent C1q are indicative of a second carbohydrate moiety whose composition resembles that of plasma glycoproteins.

The molecular weight of undissociated subcomponent C1q has been estimated by two methods: gel filtration on a calibrated Ultrogel AcA 34 column and in the ultracentrifuge by sedimentation equilibrium. The apparent molecular weight determined by gel filtration on Ultrogel AcA 34 in a buffer of conductivity 21 mmho/cm was found to be in the range 340000–360000. This is lower than the mol.wt. found by sedimentation equilibrium (430000 $\pm$ 15000). The value of 430000 agrees with results by similar methods for human and rabbit subcomponent C1q (Reid *et al.*, 1972; Calcott & Muller-Eberhard, 1972). Salt concentration appears to affect the apparent molecular weight of subcomponent C1q determined by gel filtration. Even lower apparent molecular weights result when the Ultrogel column is run in buffers of higher salt concentration. The reason for this anomalous behaviour is not understood, but it is important for the purification process that the sample and column should be at the specified buffer concentration in order to achieve separation of subcomponent C1q from other proteins.

SDS/polyacrylamide-gel electrophoresis under non-reducing conditions (Fig. 3) yielded two bands, of mol.wts. 69000 and 62000 in a molar ratio of 2:1. Under reducing conditions the mol.wts. of the bands were 30000 and 25000 in a molar ratio of 1:2. The molecular weight of the 62000-mol.wt. subunit is slightly higher than the corresponding human subunit (54000) and could be due to a difference in carbohydrate content. Eluting the 69000 and 62000 bands from the gels and re-running them under reducing conditions showed that the 69000-mol.wt. subunit is composed of two polypeptide chains of mol.wts. 30000 and 25000 (Fig. 3), and the 62000-mol.wt. subunit is composed of two polypeptide chains of mol.wt. 25000. The molecular weights of the chains calculated from SDS/polyacrylamide-gel electrophoresis have probably been overestimated owing to the influence that carbohydrate and collagen-like portions have on the rate of migration of proteins through these gels. The presence of carbohydrate in proteins tends to lead to an overestimation of molecular weights; also, the chains of collagen and peptides derived from them migrate at a much lower rate than do the globular proteins of the same molecular weight (Furthmayr & Timpl, 1971).

The results of our molecular-weight measurements suggest that bovine subcomponent C1q is similar in subunit and polypeptide structure to human C1q. The model proposed by Reid & Porter (1976) contains six identical subunits each containing three different polpeptide chains of very similar molecular weight, with chains linked in pairs by disulphide bridges, two different chains (A and B) paired in one subunit, the third chain (C) paired to an identical chain in another subunit. Our 69000-mol.wt. species corresponds to the A–B dimer, and the 62000-mol.wt. species to the C–C dimer. On reduction, the 69000-mol.wt. species gives an A-chain of mol.wt. 30000 and a B-chain of mol.wt. 25000; the C-dimer becomes two chains of mol.wt. 25000. This interpretation is consistent with the 1:2 molar ratio found for the 30000-mol.wt. (chain A) and 25000-mol.wt. (chains B and C) chains by reduction of the complete molecule. After allowing for the anomalous behaviour of collagen-like peptides, and the occurrence of carbohydrate, we conclude that bovine C1q has the same chain structure as human C1q.

The collagenase-digestion experiment (Figs. 4 and 5) indicates that there is a distinct collagen-like region in subcomponent C1q that is essential for haemolytic activity. The amino acid composition of bovine subcomponent C1q showing a high glycine value and the presence of hydroxylysine and hydroxyproline is also indicative of a collagen-like region. Digestion of subcomponent C1q by collagenase appeared to degrade the 62000-mol.wt. subunit in preference to the 69000-mol.wt. subunit. After 6h of incubation at 37°C, 70% of the 62000-mol.wt. subunit had been digested, whereas only 30% of the 69000-mol.wt. subunit had been degraded. The lower-mol.-wt. subunit (54000) of human subcomponent C1q also appears to be more susceptible to digestion by collagenase (Knobel *et al.*, 1974). After 2h the smaller subunit appeared to be completely degraded, whereas 5–10% of the larger subunit was still present after 6h of incubation.

From the molecular characteristics of human and bovine subcomponent C1q, it can be seen that these two molecules are very similar. The collagen-like properties of bovine subcomponent C1q give further support to the suggestion that it has a similar structure to that proposed for the human molecule. Reid & Porter (1976) suggest, from chemical and electron-microscope evidence, that all three polypeptide chains contain a region of collagen-like sequence that is assembled into a triple helix, with the rest of the polypeptide chains forming a globular unit at one end of the collagen helix. The collagen helices come together at the other end to generate a fibril-like assembly. This results in an appearance in the electron microscope of a 'bowl of tulips' (Knobel *et al.*, 1975), the six subunits having globular heads, the flowers, with collagen triple helices, the stems, coming together to form the fibril or pot-like structure at the base.

The haemolytic activity and the antigenic reactivity of bovine subcomponent C1q and human subcomponent C1q have been compared. When bovine subcomponent C1q was incubated in the haemolytic assay with both human subcomponents C1r and C1s (in molar proportions of 1:4:4), 95% of the total activity generated by the human subcomponents assayed together could be detected (Table 3). No activity was detected if any one of the subcomponents was omitted. Therefore it appears that bovine subcomponent C1q can take the place of human subcomponent C1q in the formation of haemolytically active component C1.

Immunochemical cross-reaction between bovine subcomponent C1q and human subcomponent C1q was shown by the formation of a spur in the precipitin pattern when commercial anti-(human subcomponent C1q) was used. No precipitin line could be detected when human subcomponent C1q was tested against our rabbit antiserum to bovine subcomponent C1q.

Subcomponent C1q is of value as a clinical diagnostic reagent for the detection of immune complexes in human plasma (Zubler & Lambert, 1977). The bovine molecule reacts in a similar way to its human counterpart and has the advantage that it can be prepared in bulk from readily available starting material.

Similarities between bovine and human complement proteins are also evident in subcomponents C1r and C1s (R. D. Campbell, N. A. Booth & J. E. Fothergill, unpublished work) and in component C4 (Booth *et al.*, 1979). Relatively large quantities of these proteins can be obtained from bovine blood, and this will allow a detailed study of the structure and activation of the classical complement pathway.

We thank Dr. S. P. Robins of the Rowett Research Institute for the determinations of the glycosylation of hydroxylysine, Dr. L. A. Fothergill for the amino acid analyses and ultracentrifugation experiments, Mrs. Jean Bathgate for excellent technical and secretarial assistance and W. Donald and Son Ltd. for their generous co-operation in the collection of blood. We are grateful to the Medical Research Council for financial support and a Research Studentship to R. D. C.

References

Aminoff, D. (1961) *Biochem. J.* **81**, 384–392

Barta, O. & Barta, V. (1972) *J. Immunol. Methods* **1**, 363–374

Beavan, G. H. & Holiday, E. R. (1952) *Adv. Protein Chem.* **7**, 319–381

Booth, N. A., Campbell, R. D. & Fothergill, J. E. (1979) *Biochem. J.* in the press.

Calcott, M. A. & Muller-Eberhard, H. J. (1972) *Biochemistry* **11**, 3443–3450

Davie, E. W., Fujikawa, K., Kurachi, K., Koide, T. & Kisiel, W. (1976) *Miami Winter Symp.* **11**, 189–202

Fong, J. S. C., Muschel, L. H. & Good, R. A. (1971*a*) *J. Immunol.* **107**, 28–32

Fong, J. S. C., Muschel, L. H. & Good, R. A. (1971*b*) *J. Immunol.* **107**, 34–40

Fothergill, J. E. & Anderson W. H. K. (1978) *Curr. Top. Cell. Regul.* **13**, 259–311

François, C., Marshall, R. D. & Neuberger, A. (1962) *Biochem. J.* **83**, 335–341

Fujikawa, K., Legaz, M. E. & Davie, E. W. (1972) *Biochemistry* **11**, 4882–4891

Fujikawa, K., Thompson, A. R., Legaz, M. E., Meyer, R. G. & Davie, E. W. (1973) *Biochemistry* **12**, 4938–4945

Furthmayr, H. & Timpl, R. (1971) *Anal. Biochem.* **41**, 510–516

Gibbons, R. A. (1966) in *Glycoproteins* (Gottschalk, A., ed.), vol. 5, pp. 29–111, Elsevier, Amsterdam, London and New York

Gigli, I., Porter, R. R. & Sim, R. B. (1976) *Biochem. J.* **157**, 541–548

Hirs, C. H. W. (1967) *Methods Enzymol.* **11**, 197–199

Immunochemistry (1970) **7**, 137–142

Isemura, M., Takahashi, K. & Ikenaka, T. (1976) *J. Biochem. (Tokyo)* **80**, 653–658

Kapitany, R. A. & Zebrowski, E. J. (1973) *Anal. Biochem.* **56**, 361–369

Kefalides, N. A. (1973) *Int. Rev. Connect. Tissue Res.* **6**, 63–107

Kisiel, W. & Davie, E. W. (1975) *Biochemistry* **14**, 4928–4934

Knobel, H. R., Heusser, C., Rodrick, M. K. & Isliker, H. (1974) *J. Immunol.* **112**, 2094–2101

Knobel, H. R., Villiger, W. & Isliker, H. (1975) *Eur. J. Biochem.* **5**, 78–82

Lamm. O. (1929) *Z. Phys. Chem. Abt. A* **143**, 177–190

Lepow, I. H., Naff, G. B., Todd, E. W., Pensky, T. & Hinz, C. F. Jr. (1963) *J. Exp. Med.* **117**, 983–1008

Lowe, D. M. & Reid, K. B. M. (1974) *Biochem. J.* **143**, 256–272

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mancini, G., Carbonara, A. O. & Heremans, J. F. (1965) *Immunochemistry* **2**, 235–254

Mayer, M. M. (1961) in *Experimental Immunochemistry* (Kabat, E. A. & Mayer, M. M., eds.), pp. 133–240, Thomas, Springfield

Moore, S. & Stein, W. H. (1963) *Methods Enzymol.* **6**, 819–831

Nelson, R. A., Jensen, J., Gigli, I. & Tamura, N. (1966) *Immunochemistry* **3**, 111–135

Ouchterlony, Ö. (1958) *Prog. Allergy* **5**, 1–78

Rapp, H. J. & Borsos, T. (1970) *Molecular Basis of Complement Action* Meredith Corp., New York

Reid, K. B. M. (1974) *Biochem. J.* **141**, 189–203

Reid, K. B. M. (1976) *Biochem. J.* **155**, 5–17

Reid, K. B. M. (1977) *Biochem. J.* **161**, 247–251

Reid, K. B. M. & Porter, R. R. (1976) *Biochem. J.* **155**, 19–23

Reid, K. B. M. Lowe, D. M. & Porter, R. R. (1972) *Biochem. J.* **130**, 749–763

Rice, C. E. & Crowson, C. N. (1950) *J. Immunol.* **65**, 201–210

Schachman, H. K. (1957) *Methods Enzymol.* **4**, 32–104

Scheidegger, J. J. (1955) *Int. Arch. Allergy* **7**, 103–110

Schmer, G., Kirby, E. P., Terrel, D. C. & Davie, E. W. (1972) *J. Biol. Chem.* **247**, 2512–2521

Sim, R. B., Porter, R. R., Reid, K. B. M. & Gigli, I. (1977) *Biochem. J.* **163**, 219–227

Spiro, R. G. (1972) *Methods Enzymol.* **28**, 1–43

Volanakis, J. E. & Stroud, R. M. (1972) *J. Immunol. Methods* **2**, 25–34

Weber, K., Pringle, J. R. & Osborn, M. (1972) *Methods Enzymol.* **26**, 2–27

Weiner, A. M., Platt, T. & Weber, K. (1972) *J. Biol. Chem.* **247**, 3243–3251

Winzler, R. J. (1955) *Methods Biochem. Anal.* **2**, 279–311

World Health Organisation (1968) *W.H.O. Bull.* **39**, 935–938

Yasmeen, D., Ellerson, J. R., Dorrington, K. J. & Painter, R. H. (1976) *J. Immunol.* **116**, 518–526

Yonemasu, K. & Stroud, R. M. (1971) *J. Immunol.* **106**, 304–313

Yonemasu, K., Stroud, R. M., Niedermeier, W. & Butler, W. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1388–1394

Ziccardi, R. J. & Cooper, N. R. (1976) *J. Immunol.* **116**, 496–503

Zubler, R. H. & Lambert, P. H. (1977) *Recent Adv. Clin. Immunol.* **1**, 125–147