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Isolation and Characterization of C1q, a Subcomponent of the First Component of Complement, from Human and Rabbit Sera

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1. C1q, a subcomponent of the first component of complement, has been isolated, in a haemolytically active and soluble form, by ion-exchange chromatography and gel filtration, from human and rabbit sera. Yields ranged from 10 to 25 mg/litre of serum and the activity of final preparations was consistently in the range 5×10^3–15×10^3 $C1qH_{50}$ units/mg. 2. The molecular weights of human and rabbit subcomponent C1q were 409600 and 417600, as determined by sedimentation equilibrium studies. 3. Subcomponent C1q from both species was shown to be composed of non-covalently linked subunits of approximately 57000 molecular weight as determined by gel-filtration or sedimentation equilibrium studies in 5.3M-guanidinium chloride. Reduction or oxidation of human and rabbit subcomponent C1q yielded three chains each having a molecular weight of approximately 23000 and which differed slightly in amino acid composition but markedly in carbohydrate content. The oxidized chains were separated, on a preparative scale, by ion-exchange chromatography in 8M-urea on DEAE-cellulose. 4. Both human and rabbit subcomponent C1q contained hydroxyproline, hydroxylysine, a high percentage of glycine and approximately 8% carbohydrate. Glutamic acid and aspartic acid were the free *N*-terminal amino acids of human subcomponent C1q whereas only serine was found in rabbit subcomponent C1q. 5. Collagenase digestion of human or rabbit subcomponent C1q caused a rapid loss of haemolytic activity which correlated with the breakdown of collagenous regions in the molecule.

The first component of complement consists of three subcomponents, C1q, C1r and C1s, which require Ca^{2+} ions to bind them together and function as a haemolytically active unit (Lepow *et al.*, 1963). The activated form of the first component of complement binds to certain classes of monomeric IgG* and 7S IgM immunoglobulins; however, the binding is strongly enhanced by aggregation of these immunoglobulins (Augener *et al.*, 1971). The binding of the first component of complement to immunoglobulin probably takes place through the C1q subcomponent (Müller-Eberhard & Kunkel, 1961). Since no enzymic activity has been detected in subcomponent C1q preparations, it has been proposed that, on binding to aggregated immunoglobulin, subcomponent C1q undergoes a conformational change which allows the activation of subcomponent C1r to take place. Subcomponent C1r has been identified as an enzyme that activates the proenzyme C1s to yield the activated form of the first component of complement (Naff & Ratnoff, 1968). Activation of the first component of complement by immunoglobulins initiates a series of reactions that utilize sequentially the other eight components of complement (Müller-Eberhard, 1968). Subcomponent C1q is therefore an important link between immunoglobulins and the complement system.

Human subcomponent C1q has been isolated previously by Müller-Eberhard (1968) by a procedure involving gel filtration, ion-exchange chromatography and Pevikon electrophoresis, and by Yonemasu & Stroud (1971) who used a precipitation procedure involving chelating agents and low-ionic-strength buffers. These reports (Müller-Eberhard, 1968; Yonemasu *et al.*, 1971) have shown that human subcomponent C1q is a glycoprotein with an unusual amino acid composition in that it contains hydroxyproline, hydroxylysine and a high percentage of glycine.

It was found that neither of the above methods was entirely satisfactory in yielding soluble, active, purified subcomponent C1q in quantities large enough for structural studies and in a form suitable for activity studies. This paper describes the isolation of human and rabbit subcomponent C1q in a highly haemolytically active and soluble form by rapid and simple chromatographic procedures. Subcomponent

* Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; $Cys(O_3H)$, cysteic acid; $Met(O_2)$, methionine sulphone.

C1q preparations from both species have a similar, and unusual, amino acid composition. Their molecular weights, subunit structure, *N*-terminal amino acids, carbohydrate content and susceptibility to digestion by collagenase are also reported.

Materials and Methods

Isolation of human and rabbit subcomponent C1q

Outdated human plasma was obtained from the Churchill Hospital, Oxford. Fresh rabbit serum was used. The plasma and serum were stored at −20°C before use. The human plasma was made 20mM with $CaCl_2$ and left to clot overnight at 4°C. After centrifugation and separation from the clot, the human serum was taken for euglobulin precipitation. All manipulations were performed at 4°C unless stated otherwise. Human and rabbit subcomponent C1q were isolated from the euglobulin precipitate of the serum essentially as described by Lepow *et al.* (1963). Serum (4 litres) was diluted eightfold in sodium acetate buffer, pH5.5, *I* 0.02, and left overnight. The euglobulin precipitate was centrifuged at 1500*g* for 30min, washed with sodium acetate buffer, pH5.5, *I* 0.02, and dissolved in 500mM-NaCl–10mM-EDTA (200ml). The solution was then centrifuged at 80000*g* for 90min. The lipid surface layer was removed and the supernatant was dialysed against two changes of 15mM-sodium phosphate–10mM-EDTA buffer, pH7.4. The dialysed sample was clarified by centrifugation at 1500*g* for 30min and applied to a column (9cm×60cm) of DEAE-cellulose equilibrated with the sodium phosphate–EDTA buffer, pH7.4. The column was eluted at the rate of 100ml/h with a hydrostatic head of 1m. Fractions of 27ml were collected. Subcomponent C1q was eluted with the starting buffer and the fractions were pooled, concentrated by ultrafiltration to about 30ml and dialysed against 16mM-sodium phosphate – 200mM-NaCl buffer, pH5.3. This solution was applied to a column (6cm×85cm) of Sephadex G-200 that was equilibrated with the sodium phosphate–NaCl buffer, pH5.3, and eluted by upward flow at a rate of 17ml/h; 17.0ml fractions were collected. The protein peak containing the subcomponent C1q haemolytic activity was eluted near the void volume of the column (Figs. 1*a* and 1*b*). This peak was pooled, concentrated by ultrafiltration to 30ml and dialysed against 430mM-sodium acetate buffer, pH5.3. This solution was chromatographed on a column (3.5cm×40cm) of CM-cellulose equilibrated with 430mM-sodium acetate buffer, pH5.2. The column was run at a flow rate of 15ml/h by using an LKB peristaltic pump; 10ml fractions were collected. After the first protein peak had been eluted (Figs. 1*c* and 1*d*) a linear gradient composed of 700ml of 430mM-sodium acetate buffer, pH5.2, and 700ml of 430mM-sodium acetate–270mM-NaCl buffer, pH5.2, was begun. The tubes containing the subcomponent C1q haemolytic activity (Figs. 1*c* and 1*d*) were pooled and concentrated and represented the finally purified subcomponent C1q.

Haemolytic assay of subcomponent C1q activity

Rabbit haemolysin was prepared from sheep erythrocyte stromata as described by Mayer (1961). The immune sera were heated at 56°C for 30min and stored at −70°C in 1.0ml samples. Whole complement was prepared by bleeding normal Hartley strain guinea pigs by cardiac puncture and allowing the blood to clot for 4 to 5h at 4°C. The pooled sera were frozen in 1.0ml samples at −70°C or used immediately in the preparation of functionally pure component C2. Functionally pure component C2 was prepared as described by Nelson *et al.* (1966). Functionally pure subcomponents C1r and C1s were prepared by ion-exchange chromatography of the solubilized euglobulin precipitate of human or rabbit serum on DEAE-cellulose as described by Lepow *et al.* (1963). These functionally purified preparations of subcomponent C1r and C1s were further purified by chromatography on Sephadex G-200 equilibrated with 16mM-sodium phosphate–200mM-NaCl buffer, pH5.3. Fractions with subcomponent C1r and C1s activity were pooled, concentrated, if necessary, by ultrafiltration and stored at −70°C in 0.5ml samples. The late components of complement (C3–C9) were supplied in the form of whole guinea-pig complement diluted 1 to 50 with 50mM-tris–40mM-HCl – 98mM-NaCl – 10mM-EDTA buffer, pH7.5. EAC'4 cells were prepared as described by Borsos & Cooper (1961).

To assay for subcomponent C1q haemolytic activity 0.5ml serial dilutions of the solution to be tested were made in 50mM-tris–40mM-HCl–0.15mM-$CaCl_2$–25mM-NaCl–146mM-sucrose buffer, pH7.5; 0.5ml of a mixture of subcomponent C1r and C1s, in the same buffer used to dilute the test sample, was added along with 0.25ml of EAC'4 cells (1.5×10^8cells/ml). After incubation at 37°C for 10min, 0.5ml of component C2 (5×10^8 effective molecules/ml) in 50mM-tris–40mM-HCl–0.15mM-$CaCl_2$ – 0.5mM-$MgCl_2$ – 25mM-NaCl – 146mM-sucrose buffer, pH7.5, was added and the cells incubated at 30°C for 15min. Then 2.0ml of whole guinea-pig complement, diluted 1 to 50 with 50mM-tris–40mM-HCl–98mM-NaCl–10mM-EDTA buffer, pH7.5, was added, to supply the components C3–C9, and then the mixture was incubated at 37°C for 90min. The cells were centrifuged, after the 90min incubation, at 1500*g* for 10min and the E_{415}^{1cm} values of the supernatants read. All appropriate controls were included in each assay. Results were expressed in H_{50} units, that is, the dilution of test material giving 50%

haemolysis of 1.50×10^8 EAC'4 cells in the presence of subcomponent C1r, and C1s and component C2 and C3–C9.

Quantitative amino acid analysis

Samples were hydrolysed, in twice-glass-distilled 5.7M-HCl, at 110±1°C, for 48h, unless indicated otherwise, in sealed evacuated tubes from which the gases had been removed by the method of Moore & Stein (1963). On completion of hydrolysis the HCl was removed by rotary evaporation at 40°C. The dried hydrolysate was dissolved in 1.2ml of 0.2M-sodium citrate buffer, pH3.5, and examined on a Beckman amino acid analyser. Where indicated 24 and 72h hydrolysis periods were used and corrections made for destruction of serine, threonine and tyrosine. Half-cystine was determined as cysteic acid after performic acid oxidation as described by Hirs (1956). Tryptophan was determined spectrophotometrically by the method of Beaven & Holiday (1952).

Carbohydrate analysis

Total hexose was determined by the orcinol–H_2SO_4 procedure of Winzler (1955) as modified by Francois *et al.* (1962). Glucosamine and galactosamine were determined on a Beckman amino acid analyser. The component C1q samples (1mg/ml) were hydrolysed under vacuum, in 4M-HCl at 110°C for 4h to release the hexosamines. The acid was removed *in vacuo* over KOH and H_2SO_4 at 4°C. Fucose was determined as described by Dische & Shettles (1948). Sialic acid was determined by the thiobarbituric acid method of Warren (1959) after hydrolysis of the subcomponent C1q samples in 0.05M-H_2SO_4 for 1h.

Polyacrylamide gel electrophoresis in sodium dodecyl sulphate

Polyacrylamide gels (5%, w/v) were made by dissolving 5g of Cyanogum 41 (mixture of 95% acrylamide monomer and 5% *NN'*-methylenebisacrylamide supplied by BDH Chemicals Ltd., Poole, Dorset, U.K.) in 95ml of 0.1M-sodium phosphate buffer, pH7.2, made 0.1% (w/v) with respect to sodium dodecyl sulphate and 0.1ml of *NN N'N'*-tetramethylethylenediamine was added. A portion (19.0ml) of this solution was mixed with 1.0ml of freshly prepared 1% (w/v) ammonium persulphate in 0.1M-sodium phosphate buffer, pH7.2, made 0.1% (w/v) with respect to sodium dodecyl sulphate. Gels (5mm×150mm) were poured. Current was applied for 15min before the sample was applied. Protein samples (0.5–2.5mg/ml), in 0.1M-tris–HCl buffer, pH8.0, made 4M with urea and 1%(w/v) with sodium dodecyl sulphate, were reduced by incubation at 37°C for 1h in the presence of 20mM-dithiothreitol. The solution was then made 40mM with iodoacetamide and incubated at 37°C for a further 20min. Unreduced protein samples were treated with 20mM-iodoacetamide to prevent disulphide interchange. Bromophenol Blue (1% w/v, 10μl) in 0.1M-sodium phosphate buffer was added to the protein solutions before application of the samples to the gels. The samples (10–50μl) were applied and the gels were subjected to electrophoresis at 8mA/gel for 6–8h until the Bromophenol Blue marker was within 1cm from the end of the gel. The gels were fixed overnight in 10% (w/v) trichloroacetic acid. Proteins were stained for 6–8h with 0.1% (w/v) Coomassie Blue and destained with methanol–acetic acid–water (21:42:340, by vol.). Carbohydrate was stained by the method of Zacharias *et al.* (1969). Rabbit IgG, bovine serum albumin, ovalbumin, pepsin, trypsin and cytochrome *c* were used as unreduced marker proteins. All these proteins, along with the three chains of human fibrinogen and the heavy and light chains of rabbit IgG were used as reduced and alkylated marker proteins. Mobilities were calculated and plotted as described by Weber & Osborne (1969). The gels were scanned for protein and carbohydrate stain by using a Joyce–Loebl microdensitometer.

Analytical ultracentrifugation

A Spinco model E ultracentrifuge was used. Sedimentation velocities were determined at 20°C by using a single-sector cell and a rotor speed of 59780rev./min for solutions in 430mM-sodium acetate buffer, pH5.2. For determinations in 5.3M-guanidinium chloride a double-sector cell was used at a rotor speed of 42040rev./min. Schlieren optics were used.

Molecular weights were determined by using the high-speed meniscus-depletion method of sedimentation equilibrium as described by Yphantis (1964). In aqueous buffer rotor speeds of between 9970 and 11590rev./min were used, and in 5.3M-guanidinium chloride a variety of speeds from 25000 to 39400rev./min were used. Raleigh interference optics were used in these sedimentation equilibrium runs. Partial specific volumes for human and rabbit subcomponent C1q were calculated from the amino acid and carbohydrate compositions.

Enzymic digestion of subcomponent C1q and its oxidized chains

Trypsin was treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone to remove chymotryptic activity as described by Kostka & Carpenter (1964). The treated trypsin (20μg in 20μl of 1mM-HCl) was added to 2mg of oxidized subcomponent

C1q which was dissolved or suspended in 0.4ml of 200mM-NH_4HCO_3 buffer, pH8.1. Digestion was carried out at 37°C for 5h. The digest was then freeze-dried, dissolved in 200μl of 200mM-NH_4HCO_3 buffer, pH6.5, and examined by electrophoresis in one dimension followed by chromatography in butanol–acetic acid–pyridine–water (15:3:10:12, by vol.) in the second dimension, either on Whatman 3MM paper or silica gel Chromogram sheets.

Subcomponent C1q (10mg) in 5.0ml of 100mM-tris–acetate buffer, pH7.4, which was 5mM with respect to calcium acetate, was incubated at 37°C with 0.5mg of collagenase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.; chromatographically purified) for 24h. Samples (50μl) were taken from the digest and control tubes at various time-intervals from 0.5 to 24h, and mixed with ice-cold buffer (0.45ml) and stored at −70°C until assayed for subcomponent C1q haemolytic activity. The precipitate and supernatant fractions of the 24h digest were separated by centrifuging at 80000*g* for 30min. Both fractions were examined on polyacrylamide gels run in sodium dodecyl sulphate and by amino acid analysis. The supernatant fraction was further examined by gel filtration on Sephadex G-200 and Sephadex G-25 in 0.1M-acetic acid.

N-terminal analysis

Qualitative *N*-terminal analyses were carried out by the dansyl method described by Gray (1967). Quantitative *N*-terminal analyses were performed by the thioacetylthioglycollic acid method of Mross & Doolittle (1971). Subcomponent C1q (5.98mg) was suspended in 0.5ml of 500mM-tris–HCl buffer, pH8.6. Thioacetylthioglycollic acid reagent (0.5ml) (240mg of thioacetylthioglycollic acid, 240μl of triethylamine made up to 4.0ml with pyridine) was added and the mixture incubated at 40°C for 60min. Excess of reagents were extracted twice with benzene (5.0ml) and three times with acetone (5.0ml). The thioacetylthioglycollic acid–protein was dried, dissolved in water (1.0ml) and freeze-dried. Trifluoroacetic acid (0.2ml) was added to the freeze-dried thioacetylthioglycollic acid–protein and the solution was incubated at 40°C for 60min. The solution was extracted twice with diethyl ether (1.5 and 1.0ml). Each extract was layered, in turn, over 0.1ml of 5.7M-HCl, in hydrolysis tubes, mixed by inversion and the ether layer was then removed by passing a stream of N_2 over the gently heated solution. Once all the ether had been removed 5.7M-HCl (0.2ml) was added and the sample hydrolysed at 110°C for 16h in the sealed tube. The hydrolysate was dried and the resulting amino acids were quantitatively analysed on a Locarte amino acid analyser which could detect 1nmol of amino acid. The remaining thioacetylthioglycollic acid–protein was dried, suspended in water (0.5ml) and thioacetylthioglycollic acid reagent added (0.5ml) and treated as above to locate the next new *N*-terminal amino acid.

Electrophoresis and chromatography

Pevikon-block electrophoresis of subcomponent C1q was performed as described by Müller-Eberhard (1968). High-voltage electrophoresis was carried out as described by Katz *et al.* (1959). Peptide samples in 100mM-NH_4HCO_3 buffer were applied as a 1cm-wide strip to Whatman 3 MM paper and were subjected to electrophoresis at 3kV for 1h by using either pH6.5 or 3.5 buffers as described by Crumpton & Wilkinson (1965). Electrophoresis and chromatography on silica-gel Chromogram sheets (type 6061; Eastman–Kodak, Kirkby, Liverpool, U.K.) was performed exactly as described by Sargent & Vadlamudi (1968). Chromatography of Dns-amino acids on polyamide sheets (type 15153, BDH Chemicals Ltd.) was for 30min in 1.5% (v/v) aq. formic acid, followed by 60min in benzene–acetic acid (9:1, v/v) in the second dimension. After examination of the plate under u.v. light, the plates were run in ethyl acetate–methanol–acetic acid (20:1:1, by vol.) for 45min, also in the second dimension. *N*-Terminal amino acids were identified by comparison with the standard Dns-amino acids. Paper chromatography was performed in butanol–acetic acid–water–pyridine (45:9:36:30, by vol.).

Preparative separation of the oxidized or reduced and alkylated chains of human subcomponent C1q

Reduced and alkylated preparations of human subcomponent C1q were fractionated on a 5% polyacrylamide gel run in 0.1M-sodium phosphate buffer, pH7.2, which was 0.1% (w/v) with respect to sodium dodecyl sulphate, by using a Brownstone preparative polyacrylamide-gel-electrophoresis apparatus (Brownstone, 1969). The run was performed by Mr. A. Brownstone, National Institute for Medical Research, Mill Hill, London. Three main fractions were obtained which corresponded to the three bands located on the analytical polyacrylamide-gel electrophoresis performed under the same conditions (Fig. 2*d*).

The procedure of Hirs (1956) was used to prepare oxidized samples of human and rabbit subcomponent C1q, which were then fractionated on DEAE-cellulose in buffers which were 8M with respect to urea. In a typical fractionation (Fig. 3) oxidized human subcomponent C1q (26mg) in 3mM-sodium phosphate–8M-urea buffer, pH7.8, was applied to a column (1.1cm × 65cm) of DEAE-cellulose equilibrated with the same buffer. After elution of the first two protein

peaks (Fig. 3) the molarity of the eluting buffer was raised to 67mM and a third protein peak eluted (Fig. 3). The same result was achieved by using a linear gradient from 3mM- to 67mM-sodium phosphate (both buffers pH7.8 and 8M with respect to urea). A flow rate of 15ml/h was used and 3.0ml fractions were collected.

Reduction and alkylation of subcomponent C1q

Complete reduction and alkylation of subcomponent C1q was performed by dissolving C1q (20mg) in 3.0ml of 6.0M-guanidinium chloride, containing 0.1M-tris–acetate buffer, pH8.0. Dithiothreitol was then added to a final concentration of 20mM and the protein solution kept at 37°C for 2h. Iodoacetic acid (2.1mol/mol of dithiothreitol) in 2M-tris base was added and the mixture left for 30min at room temperature. The mixture was then dialysed against ice-cold water (5 litres) and finally against 0.2M-acetic acid, before being freeze-dried.

Preparation of antisera

Purified human and rabbit subcomponent C1q (1.0mg/ml in 140mM-NaCl) were mixed with an equal volume of Freund's complete adjuvant. These mixtures (2.0ml), of adjuvant plus human subcomponent C1q, and adjuvant plus rabbit subcomponent C1q, were injected subcutaneously into four sites in rabbits, and six sites in guinea pigs, respectively. After 4 weeks the rabbits were given an intravenous booster injection of 1.0ml of alum-precipitated human subcomponent C1q (0.5mg/ml of suspension) and the guinea pigs were given a further six subcutaneous injections of alum-precipitated rabbit subcomponent C1q (1.0ml of 0.5mg/ml of suspension). After the fifth week the animals were bled at weekly intervals.

Results

Purification of human and rabbit subcomponent C1q

Chromatography of the solubilized euglobulin precipitate from human or rabbit serum on DEAE-cellulose gave two protein peaks. The first peak, which was equivalent to approximately 14% of the applied protein, contained all the subcomponent C1q haemolytic activity. The partially purified subcomponent C1q was then gel filtered on Sephadex G-200 to remove low-molecular-weight contaminants, which consisted mainly of IgC (Figs. 1*a* and 1*b*); 27 and 15% respectively of the partially purified human and rabbit subcomponent C1q applied to the column was eluted, with all the haemolytic activity near the void volume of the column (Figs. 1*a* and 1*b*). The recovery of haemolytic activity at this step varied from 88 to 112% of that applied to the column. Final purification was achieved by ion-exchange chromatography on CM-cellulose. Approximately 65 and 32% respectively, of the partially purified human and rabbit subcomponent C1q applied to the column was eluted at I 0.180–0.230, along with all haemolytic activity (Figs. 1*c* and 1*d*). The recovery of haemolytic activity at this step was 90 to 110% of that applied to the column.

The yield of human subcomponent C1q from outdated human plasma was approximately 25mg/l, whereas the yield of rabbit subcomponent C1q from fresh frozen rabbit serum varied from 10 to 15mg/l. The specific haemolytic activities of both human and rabbit component C1q, in the assay system used, were of the same order but varied from preparation to preparation from 5×10^3 to 15×10^3 $C1qH_{50}$ units/mg.

The threefold variation in specific activity between different final preparations of subcomponent C1q is due to differences in the sensitivity to haemolysis of the EAC'4 cells used, since they had to be prepared weekly with different batches of fresh sheep erythrocytes. For accurate comparison of the specific activities of human and rabbit subcomponent C1q or partially purified fractions of subcomponent C1q all the samples were tested with the same batch of EAC'4 cells.

Electrophoresis on Pevikon

Both human and rabbit subcomponent C1q migrated as a single sharp protein band along with all the haemolytic activity towards the cathode on electrophoresis at 4°C, pH6.0 and I 0.1. However, this procedure resulted in large losses of both protein and haemolytic activity. Approximately 50% of both the applied material and haemolytic activity was usually recovered.

Sedimentation velocity

Both human and rabbit subcomponent C1q, in acetate buffer, pH5.2, I 0.150, behaved as single components in the analytical ultracentrifuge. The $s_{20,w}$ varied, linearly, with the protein concentration from 9.15S at 4.64mg/ml to 9.92S at 1.16mg/ml. On extrapolation to zero concentration an $s^0_{20,w}$ of 10.20S was obtained for both human and rabbit subcomponent C1q. Rabbit IgG run under the same conditions, at a concentration of 1.3mg/ml had an $s_{20,w}$ of 6.68S.

Immunodiffusion

Human subcomponent C1q gave a single line against rabbit anti-(human C1q), and also against rabbit anti-(human whole serum), in Ouchterlony double-diffusion done in agarose gels. A single precipitin arc was obtained in immunoelectrophoresis

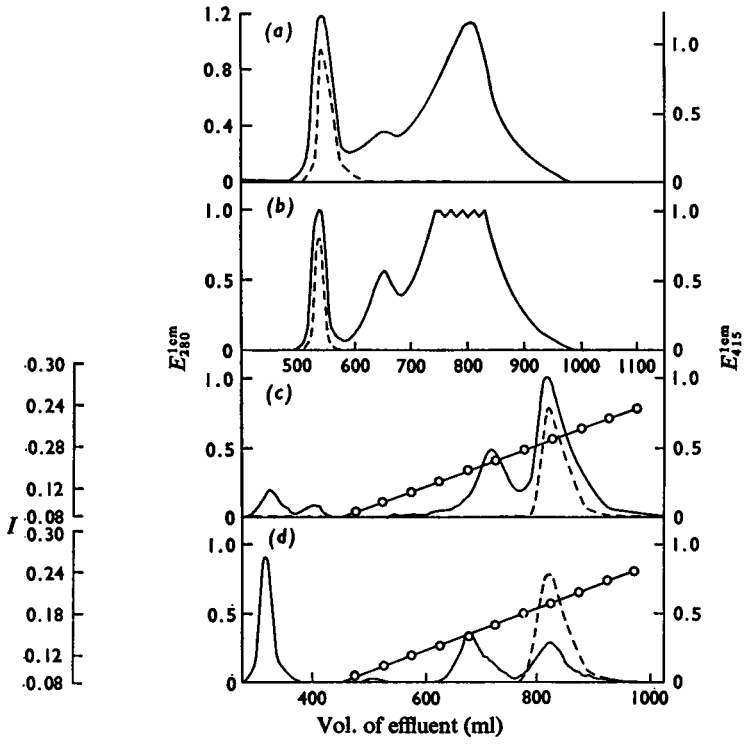

Fig. 1. *Purification of subcomponent C1q on Sephadex G-200 and CM-cellulose*

Partially purified human (*a*) and rabbit (*b*) subcomponent C1q preparations were fractionated on Sephadex G-200, and then further purified on CM-cellulose (*c* and *d*, respectively). ——, E^{1cm}_{280}; ----, E^{1cm}_{415} (indicating the subcomponent C1q haemolytic activity); o, *I* (ionic strength). Full experimental details are given in the text.

of anti-(human C1q) with purified subcomponent C1q. Both the precipitin line and arc were abolished by heating the subcomponent C1q at 56°C for 30min beforehand. Rabbit subcomponent C1q gave one major and one minor precipitin line in Ouchterlony double-diffusion against guinea-pig anti-(rabbit C1q) and guinea pig anti-(rabbit whole serum). The major line disappeared on heating the rabbit subcomponent C1q at 56°C for 30min. The minor precipitin line was due to the presence of a small amount of the protein eluted before rabbit subcomponent C1q on CM-cellulose (Fig. 1*d*). Human subcomponent C1q gave no detectable precipitin line when tested against guinea-pig anti-(rabbit C1q).

Effect of heat treatment on C1q

Over 93% of the human subcomponent C1q, and over 90% of the rabbit subcomponent C1q, was precipitated by heating at 56°C for 30min, in both cases causing complete loss of haemolytic activity.

Extinction coefficient of subcomponent C1q

The $E^{1\%}_{1cm}$ of human subcomponent C1q in 0.5M-acetic acid was 6.82 at 280nm; the concentration of the subcomponent C1q solution was determined by drying to constant weight. The above extinction coefficient was used in calculations involving rabbit C1q as well as those involving human subcomponent C1q.

Amino acid and carbohydrate composition of subcomponent C1q

The amino acid compositions of human and rabbit subcomponent C1q are given in Table 1. The overall compositions are very similar and notable features are the high glycine values and the presence of hydroxylysine and hydroxyproline. The most significant difference between the human and rabbit subcomponent C1q compositions is in the histidine content, which is higher by almost a factor of two in rabbit C1q. Human and rabbit subcomponent C1q

Table 1. *Amino acid compositions of human and rabbit subcomponent C1q*

Samples of human subcomponent C1q (0.382mg) and rabbit subcomponent C1q (0.187mg) were hydrolysed for 24, 48 and 72h. Experimental details are given in the text. Results are expressed as amino acid residues/100 residues and mol of amino acid per mol of protein (taking molecular weights of 409000 and 417000 respectively for human and rabbit component C1q).

	Amino acid composition					
	μmol (corrected)		Residues/100 residues		mol/mol of protein	
Amino acid	Human	Rabbit	Human	Rabbit	Human	Rabbit
Hyl	0.058	0.019	2.09	1.43	62	42
Lys	0.112	0.047	4.04	3.58	120	105
His	0.039	0.033	1.41	2.52	42	74
Arg	0.126	0.072	4.55	5.46	135	162
Hyp	0.125	0.065	4.51	4.93	134	145
Asp	0.227	0.111	8.19	8.38	244	246
Thr	0.146	0.055	5.62	4.13	156	128
Ser	0.131	0.062	4.72	4.71	140	139
Glu	0.233	0.113	8.39	8.54	249	251
Pro	0.192	0.085	7.05	6.40	206	188
Gly	0.475	0.233	17.09	17.63	509	519
Ala	0.121	0.074	4.36	5.59	130	164
Cys	0.048	0.033	1.73	2.46	52	72
Val	0.165	0.078	5.95	5.93	177	174
Met	0.043	0.013	1.55	1.01	46	30
Ile	0.118	0.045	4.26	3.41	126	101
Leu	0.172	0.076	6.20	5.76	184	170
Tyr	0.106	0.036	3.57	2.75	114	81
Phe	0.117	0.063	4.23	4.80	126	141
Trp	0.014	0.008	0.50	0.58	15	17

Table 2. *Carbohydrate compositions of human and rabbit subcomponent C1q*

Full experimental details are given in the text.

	Carbohydrate (g/100g of protein)	
	Human	Rabbit
Hexose	6.95	6.78
Hexosamine	0.65	0.57
Fucose	0.11	0.10
Sialic acid	0.32	0.12
Total	8.03	7.57

have approximately the same amount of carbohydrate and most of it is in the form of neutral hexoses (Table 2); 90% of the hexosamine in both human and rabbit subcomponent C1q was glucosamine.

Terminal amino acids

N-Terminal analysis by the dansyl method showed that glutamic acid and aspartic acid were present as *N*-terminal amino acids in human subcomponent C1q, whereas only *N*-terminal serine was found in rabbit subcomponent C1q. A quantitative determination of the *N*-terminal amino acids in human subcomponent C1q was made by the thioacetylthioglycollic acid method of Mross & Doolittle (1971); 62.2nmol of aspartic acid, 60.7nmol of glutamic acid, 9nmol of glycine and less than 3nmol of any other amino acid per 5.98mg (which is 14.63nmol taking a molecular weight of 409000 for human C1q) of human subcomponent C1q was found. The yield of *N*-terminal aspartic acid from a Bence-Jones protein, by the thioacetylthioglycollic acid method, was 65% of that expected. After making corrections, based on the yield of *N*-terminal amino acid obtained from the Bence-Jones protein, the thioacetylthioglycollic acid *N*-terminal analysis results show that 6.5mol of *N*-terminal glutamic acid and 6.4mol of *N*-terminal aspartic acid are present per mol of human subcomponent C1q. It should be noted, as described below, that one of the three chains of human subcomponent C1q appears to have no free *N*-terminal amino acid.

Molecular weights of subcomponent C1q, the non-covalently linked subunits of C1q and the peptide chains of C1q

In non-dissociating conditions, sedimentation-equilibrium studies performed according to the high-speed meniscus-depletion method (Yphantis, 1964), gave molecular weights of 409000±5000 and 417000±29000, respectively, for human and rabbit subcomponent C1q (Table 3). Each of these results was the average of five runs done at different speeds of from 9945 to 13550rev./min.

Three different techniques were used to examine, under dissociating conditions, the molecular weights of the non-covalently linked subunits, and covalently linked peptide chains, of subcomponent C1q. They were: (i) polyacrylamide-gel electrophoresis in sodium dodecyl sulphate on 5% gels; (ii) gel filtration in 5.3M-guanidinium chloride, pH3.0, on Sephadex G-200; (iii) sedimentation-equilibrium studies in 5.3M-guanidinium chloride done by the high-speed meniscus-depletion method. The results are summarized in Table 3.

(i) Apparent molecular weights of 69700 and 69200 were obtained for unreduced preparations of human and rabbit subcomponent C1q by using unreduced globular proteins as molecular-weight markers. Values of 53000 and 52000 were obtained if reduced and alkylated globular proteins were used as molecular-weight markers (Table 3). The unreduced human subcomponent C1q preparations consistently gave one major band on the 5% polyacrylamide gels, under the conditions used (Fig. 2*a*). This band accounted for greater than 90% of the protein stain on the gel as estimated from the densitometer scans (Fig. 2*a*). Unreduced rabbit subcomponent C1q preparations always showed significant amounts of another component of lower apparent molecular weight (Fig. 2*b*). The apparent molecular weight of this component was 62400 with unreduced marker proteins and 47000 with reduced marker proteins. The ratio of the 69200 unreduced rabbit C1q component to the 62400 component, as estimated from the densitometer scans of gels stained for protein, varied from 1.3:1.0 (Fig. 2*b*) to 3.56:1.0 (Fig. 2*c*) in different preparations. The 62400 component stained less heavily for carbohydrate than the 69200 component (Fig. 2*b*). The ratio of the carbohydrate contents, as estimated from the densitometer scans, was 3.24:1.0 (Fig. 2*b*) in a preparation where the ratio of protein contents was 1.3:1.0 (Fig. 2*b*). The presence of different amounts of carbohydrate on these two components of unreduced rabbit subcomponent C1q and the variability in amounts of the lower-molecular-weight component suggest that it may be derived from the higher-molecular-weight component.

Reduction and alkylation, or performic acid oxidation, of human subcomponent C1q gave three poly-

Table 3. *Molecular weights of human and rabbit subcomponent C1q, their noncovalently linked subunits and covalently linked peptide chains*

Full experimental details are given in the Text.

	Molecular weights					
Method ...	5% Polyacrylamide gels run in sodium dodecyl sulphate		Sephadex G-200 gel filtration		Sedimentation-equilibrium studies (weight average)	
	Human	Rabbit	Human	Rabbit	Human	Rabbit
Unreduced						
Non-dissociating conditions	*	*	*	*	409000	417000
Dissociating conditions	69700† (or 53000)	69200† (or 52000)	57500† (or 59600)	*	57000	56000
Totally reduced or oxidized						
Dissociating conditions	Chain A 34800	32000			Chain A 23500 *	25100
	Chain B 31600	32000	22800	23000	Chain B * *	(mixture of
	Chain C 27500	30000	(mixture of three chains)		Chain C 27000 26800	three chains)

* Not estimated.
† Determined by using unreduced marker proteins.

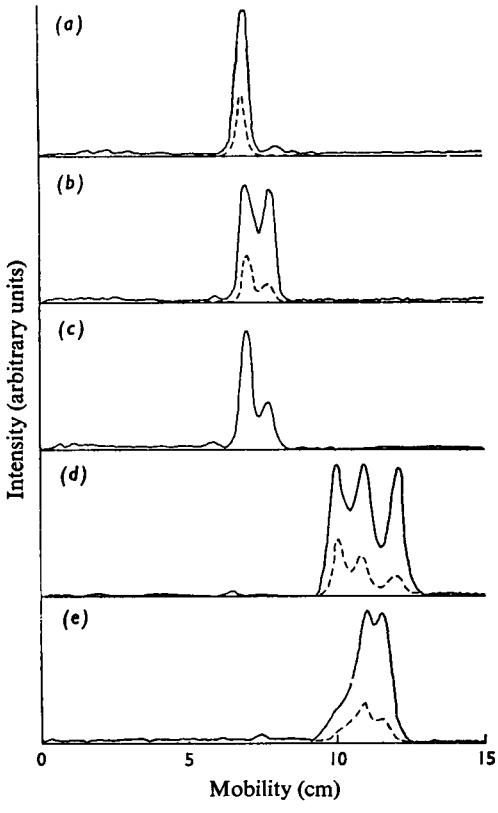

Fig. 2. *Densitometer scans of polyacrylamide-gel electrophoresis of subcomponent C1q run on 5% gels in sodium dodecyl sulphate*

(*a*) Unreduced human subcomponent C1q; (*b*) unreduced rabbit subcomponent C1q; (*c*) unreduced rabbit subcomponent C1q; (*d*) reduced and alkylated human subcomponent C1q; (*e*) reduced and alkylated rabbit subcomponent C1q. ——, Protein; ----, carbohydrate. Full experimental details are given in the text.

peptide chains of apparent molecular weights 34800, 31600 and 27500 when run on 5% polyacrylamide gels in sodium dodecyl sulphate (Fig. 2*d*). The proportions of protein stain and carbohydrate stain in each band, respectively, were 1.07:1.07:1.00, and 4.66:2.68:1.00, taking the 27500 band as 1.00 in both cases (Fig. 2*d*). The effect that the apparently different carbohydrate contents of each chain may have on the determination of their molecular weights on polyacrylamide gels in sodium dodecyl sulphate is considered in the Discussion section. Reduction and alkylation of rabbit subcomponent C1q preparations, of the type which behave as shown in Fig. 2(*b*) without reduction, yielded two poorly separated bands of apparent molecular weights 32000 and 30000. The ratios of protein and carbohydrate, as estimated from densitometer scans of the appropriately stained gels, were 1.30:1.00 and approximately 2.16:1.00, taking the 30000 band as 1.00 in both cases. Performic acid oxidation of rabbit subcomponent C1q, followed by electrophoresis on 5% polyacrylamide gels in sodium dodecyl sulphate, showed that the 32000 material was composed of two components that ran together in their reduced and alkylated forms.

(ii) Unreduced human subcomponent C1q gave two peaks when run on Sephadex G-200 in 5.3M-guanidinium chloride. The first peak was eluted in the void volume and corresponded to aggregated material of greater than 200000 molecular weight. The second peak was eluted at a position corresponding to a molecular weight of 57500 or 59600 depending on whether unreduced, or reduced and alkylated, markers were used. This second peak was very symmetrical, suggesting the presence of only one component, on a size basis. On concentrating and rerunning the second 57500-molecular-weight peak, two peaks were again observed and eluted at positions corresponding to greater than 200000 in molecular weight and 57500. All peaks eluted from Sephadex G-200 in 5.3M-guanidinium chloride yielded the same expected unreduced and reduced patterns on polyacrylamide gels run in sodium dodecyl sulphate. Both human and rabbit subcomponent C1q on reduction and alkylation or oxidation gave a single symmetrical peak of apparent molecular weight 23000 when gel filtered on Sephadex G-200 in 5.3M-guanidinium chloride.

(iii) Sedimentation-equilibrium studies by the high-speed meniscus-depletion method (Yphantis, 1964) in 5.3M-guanidinium chloride at 28000rev./min gave molecular weights of 57000 and 56000 respectively for unreduced human and rabbit subcomponent C1q. The human preparation examined was a sample of the peak eluted, at a position corresponding to a molecular weight of 57500, from Sephadex G-200 run in 5.3M-guanidinium chloride. Bovine serum albumin run under the same conditions had an apparent molecular weight of 71000. Studies on the oxidized chains of human and rabbit subcomponent C1q, which were separated as described below, gave values ranging from 23500 for the human A chain to 27000 for the human C chain (Table 3). The oxidized chains were examined at 39400rev./min. The reduced and alkylated light chain of rabbit IgG run under the same conditions had an apparent molecular weight of 24000.

Separation of performic acid-oxidized chains

Both human and rabbit subcomponent C1q separated into three fractions after performic acid

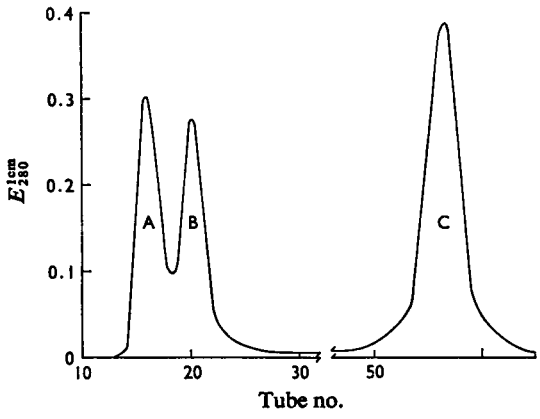

Fig. 3. *Separation of the oxidized chains of human subcomponent C1q on DEAE-cellulose*

Oxidized human subcomponent C1q (26mg) was applied to a column (1.1cm×65cm) of DEAE-cellulose and eluted with 3mM-sodium phosphate–8M-urea buffer, pH7.8, up to tube 36 and 67mM-sodium phosphate–8M-urea buffer, pH7.8, after tube 36. ——, E^{1cm}_{280}. Full experimental details are given in the text.

oxidation and chromatography on DEAE-cellulose in sodium phosphate buffer, pH7.8, *I* 0.003 and 8M with respect to urea (Fig. 3). The first two fractions, A and B, separated on the column without the use of a salt gradient (Fig. 3). The third fraction, C, was eluted by a linear salt gradient or, simply, in a stepwise manner with sodium phosphate buffer, pH7.8, *I* 0.08 and 8M with respect to urea. A single protein peak was obtained by both methods. These fractions A, B and C corresponded to the three bands observed on the 5% polyacrylamide gels run in sodium dodecyl sulphate (Fig. 2*d*). Human fraction A corresponded to the slowest-moving band on the polyacrylamide gel which had a relatively high carbohydrate content (Fig. 2*d*) and an apparent molecular weight of 34800. The human B and C fractions corresponded to the bands of apparent molecular weights 31600 and 27500 respectively (Fig. 2*d*). The rabbit A and B fractions corresponded to the poorly resolved slow-moving bands on the polyacrylamide gels, which had an apparent molecular weight of approximately 32000, whereas the rabbit C fraction corresponded to the 30000-molecular-weight band.

The yields of fractions A, B and C in a typical experiment were 27, 28 and 34% respectively of the total protein applied to the column (Fig. 3). This is very close to the yields expected if there were equimolar amounts of each chain and each chain was approximately the same molecular weight. In some experiments, however, the total recovery from the DEAE-cellulose column was low and the yields of the A and B chains in these experiments were low. Thus, recoveries of 16, 20 and 35% respectively for the A, B and C chains were observed in one experiment.

Amino acid and N-terminal analyses of the oxidized A, B and C chains

The amino acid analyses of the human subcomponent C1q performic acid-oxidized A, B and C chains isolated from the DEAE-cellulose column are given in Table 4. Similar amino acid analyses were obtained for the reduced and alkylated chains of human subcomponent C1q prepared either by elution of each chain directly from several analytical polyacrylamide gels, or by separation of the chains by fractionation of the mixture on a preparative polyacrylamide gel system (Brownstone, 1969). The number of amino acid residues per mol of each chain was calculated on consideration of the number of histidine-staining peptides found per chain and a probable molecular weight of about 23000 per chain.

N-Terminal analysis of the human A and C chains by the thioacetylthioglycollic acid method showed that the *N*-terminal sequence of the A chain was Glx-Asx-Leu and that the *N*-terminal amino acid of the C chain was Asx. The yield of amino acid at each of these steps was approximately 40% of that expected assuming that the A and C chains have molecular weights of approximately 23000. The human B chain yielded no significant amount of free *N*-terminal amino acid when examined by the thioacetylthioglycollic acid method or the dansyl method, but small amounts of glutamic acid were detected by both methods. Serine was found to be the *N*-terminal amino acid of the rabbit C chain by the dansyl method.

Tryptic digestion of performic acid-oxidized subcomponent C1q and its separated A, B and C chains

Oxidized human and rabbit subcomponent C1q both gave 33 or 34 soluble tryptic peptides when examined, on paper, by a two-dimensional technique of electrophoresis in one dimension followed by chromatography in the other. The general distribution of peptides in the two 'maps' was similar but there appeared to be few common peptides. When a tryptic digest of oxidized human subcomponent C1q was examined on thin-layer sheets by using the same two-dimensional procedure 30 peptides were located. Of these 30 peptides, 19 were found in the A chain, 12 in the B chain and 12 to 13 in the C chain. The B and C chains gave different peptide 'maps' and both contained peptides which were not found in the tryptic digest of the A chain. This was best illustrated by staining for histidine which showed that the A chain

Table 4. *Amino acid compositions of the oxidized chains of human subcomponent C1q*

Experimental details are given in the text. The results are expressed as residues/100 residues, and mol/mol assuming the integral number of histidine residues shown below. Residues/mol for the B chain are shown assuming histidine equal to two or three residues.

	Amino acid composition						
	Residues/100 residues			mol/mol, assuming:			
Amino acid	Chain A	Chain B	Chain C	His, 2.00 Chain A	His, 2.00 Chain B	His, 3.00 Chain B	His, 4.00 Chain C
Hyl	1.99	2.39	1.49	3.80	3.46	5.21	2.61
Lys	3.45	3.66	3.92	6.60	5.30	7.99	6.89
His	1.05	1.38	2.28	2.00	2.00	3.00	4.00
Arg	5.88	4.34	3.60	11.20	6.30	9.40	6.31
Hyp	4.39	3.02	5.49	8.40	4.36	6.59	9.65
Asp	7.78	9.22	8.01	14.80	13.39	20.06	14.01
Thr	3.76	5.82	5.09	7.20	8.42	12.60	8.93
Ser	6.12	4.30	5.26	11.70	6.21	9.37	9.21
Glu	10.65	8.41	8.30	20.40	12.20	18.30	14.59
Pro	6.60	6.09	6.58	12.60	8.81	13.22	11.55
Gly	17.60	18.20	17.19	33.70	27.00	39.98	30.00
Ala	4.30	5.62	3.91	7.70	8.18	12.21	6.89
$Cys(O_3H)$	1.20	1.52	1.68	2.30	2.21	3.31	3.94
Val	6.60	5.00	7.17	12.60	7.28	10.81	12.60
$Met(O_2)$	1.10	2.15	1.82	1.90	3.12	4.66	3.16
Ile	4.35	4.15	3.56	8.30	6.53	9.82	6.24
Leu	5.98	6.20	6.62	11.40	8.95	13.45	11.61
Tyr	2.98	3.10	3.85	5.70	4.50	6.74	6.76
Phe	4.50	4.97	4.15	8.60	7.21	10.85	7.28
Number of amino acids				191	145	217	175
Estimated molecular weight				23500	17300	25800	20000
Number of trypsin susceptible bonds				22	15	23	16
Number of tryptic peptides found				19	12	12	13

had two peptides which stained strongly for histidine, the B chain showed three or four and the C chain showed four or five. The three chains appeared to have approximately six or seven common, or closely related, peptides on comparing the 'maps'; however, the presence of different amounts of carbohydrate on each chain made interpretation of these maps difficult.

Collagenase digestion

Digestion of human and rabbit subcomponent C1q with collagenase at 37°C caused a rapid loss of haemolytic activity (Fig. 4). Control samples of subcomponent C1q left at 37°C for over 24h showed no significant loss of activity (Fig. 4). Incubation of bovine serum albumin with collagenase under the same digestion conditions gave no indication of degradation of the bovine serum albumin by traces of other proteolytic enzymes that might have been present as contaminants in the collagenase preparation. The loss of haemolytic activity, during digestion, correlated with the appearance of an insoluble residue which contained no hydroxylysine or hydroxyproline and had a relatively low glycine content (Table 5). This residue, which was approximately 60% of the digest, was a mixture of at least four or five peptide chains with apparent molecular weights ranging from 10000 to 6000 as determined by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate. The soluble portion of the digest was gel filtered on Sephadex G-200 in 0.1 M-acetic acid. All the applied material was eluted in a single broad peak corresponding to the elution position of low-molecular-weight peptides. This material was richer in hydroxyproline, proline, hydroxylysine and glycine than the intact subcomponent C1q (Table 5).

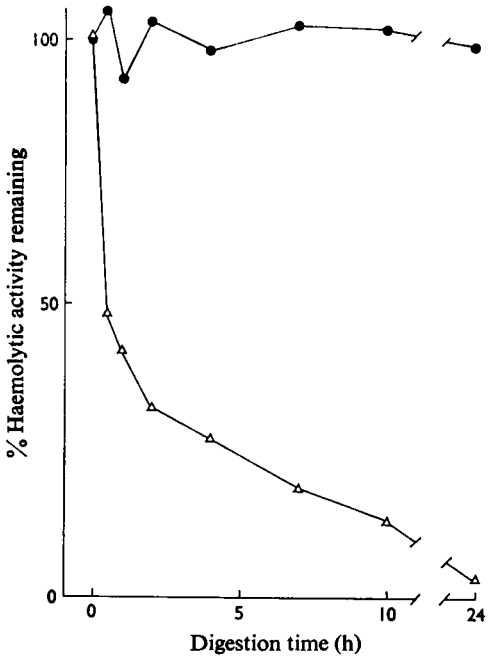

Fig. 4. *Collagenase digestion of human subcomponent C1q*

Human subcomponent C1q (1100 $C1qH_{50}$ units/ml) was digested with collagenase as described in the text. Samples were removed at times from 0.5 to 24h, and frozen at −70°C until required for haemolytic activity determination. The percentage of the remaining haemolytic activity is plotted against time of digestion. △, Collagenase digest; ●, control without collagenase.

Table 5. *Amino acid compositions of the precipitates and supernatants of the collagenase digests of human and rabbit subcomponent C1q*

Full experimental details are given in the text. The results are expressed as amino acid residues/100 residues.

Amino acid	Amino acid composition (residues/100 residues)			
	Precipitate		Supernatant	
	Human	Rabbit	Human	Rabbit
Hyl	0.21	0.49	4.69	3.06
Lys	4.38	4.18	2.40	3.75
His	2.28	4.21	0.01	2.08
Arg	3.93	4.73	5.71	5.59
Hyp	Nil	Nil	13.94	8.00
Asp	11.19	10.13	6.19	6.59
Thr	5.78	6.78	3.65	3.76
Ser	5.31	5.10	1.09	3.53
Glu	10.32	8.31	8.85	7.78
Pro	4.28	4.65	5.25	8.72
Gly	12.07	9.80	23.50	22.85
Ala	4.17	4.81	6.00	6.85
Cys	1.31	*	1.21	*
Val	11.40	12.48	0.47	6.00
Met	1.67	1.06	0.84	0.62
Ile	2.94	2.54	7.90	4.07
Leu	7.92	7.87	6.10	5.12
Tyr	4.52	5.64	1.76	0.76
Phe	6.58	7.23	0.23	1.50

* Not estimated.

Discussion

The methods used in this paper yield highly purified human and rabbit subcomponent C1q in a soluble and haemolytically active form. It is important to have highly purified subcomponent C1q preparations for structural studies and the importance of having soluble haemolytically active preparations to investigate the relationship between the structure of subcomponent C1q and its activity is most readily illustrated by the collagenase digestion experiments, from which it can be concluded that cleavage of collagenous regions of the molecule causes a rapid loss of haemolytic activity (Fig. 4, Table 5).

The human and rabbit subcomponent C1q preparations have very similar amino acid and carbohydrate compositions (Tables 1 and 2). Human subcomponent C1q, prepared by a precipitation method employing chelating agents and low-ionic-strength buffers also shows a similar amino acid and carbohydrate composition (Yonemasu *et al.*, 1971), suggesting that the final product in both preparations is essentially the same. Müller-Eberhard (1971) has also reported a partial analysis of human subcomponent C1q which agrees well with the results given here and by Yonemasu *et al.* (1971). The most striking difference in composition between human and rabbit subcomponent C1q is the much-higher, almost twofold, histidine content of rabbit C1q (Table 1). Despite the otherwise overall similarity in amino acid compositions, human and rabbit subcomponent C1q appeared to have very few common peptides when peptide 'maps' of tryptic digests of the two species were compared. In both cases a total of 33 to 34 peptides were found. Taking into account the potentially susceptible bonds available (Table 1) a total of 34 peptides is the pattern that might be expected to be derived from three peptide chains of approximately 23000 molecular weight each, which had several peptides in common. Peptide 'maps' of tryptic digests of the separated oxidized chains supported this view

although it must be remembered that the presence of different amounts of carbohydrate on each chain makes interpretation of these peptide 'maps' difficult. The amino acid analysis of the performic acid-oxidized A, B and C chains (Table 4) show quite clearly that each chain has a very similar, overall, composition to the whole subcomponent C1q. Apart from the peptide 'maps', several facts suggest that these three chains, though similar, are distinct and not, for example, derived by limited proteolytic digestion from one chain. The molecular weights of each of the chains appears to be very similar (Table 3). Therefore it is difficult to envisage how chains B and C could be derived from chain A, especially when their percentages of arginine, histidine, threonine and methionine are compared (Table 4). The presence of constant percentages of glycine and proline and approximately equal percentages of hydroxylysine and hydroxyproline is very suggestive of there being discrete collagenous regions present in each chain. Expressing the amino acid compositions of the A, B and C chains in the form of residues/mol, histidine being taken as two residues in the A chain, two or three in the B chain, four in the C chain, yields estimated molecular weights in the range of 25800–20000 which agrees with the gel-filtration and sedimentation equilibrium results (Table 3). The molecular weight of the B chain of 17300 estimated by assuming a value of two for histidine is probably too low, since three peptides stained strongly for histidine in the 'maps' of the tryptic digest. It has been included in Table 4 for comparison with the values obtained when histidine is taken as three since, in this case, the estimated molecular weight was unexpectedly higher than that of the A chain. The *N*-terminal analyses of the whole human subcomponent C1q and of its separated oxidized chains are consistent with there being three discrete chains present in equimolar amounts. Equimolar amounts of *N*-terminal glutamic acid and aspartic acid were obtained, by the thioacetylthioglycollic acid method, each in one-third of the expected yield of total *N*-terminal amino acid, based on a recovery of 65% and an average chain length of 23000. Both the thioacetylthioglycollic acid and dansyl methods showed that glutamic acid and aspartic acid were the *N*-terminal amino acids of the A and C chains respectively and that the B chain had no detectable free *N*-terminal amino acid. These observations suggest the presence of an equimolar amount of each chain. The proportion of the three chains, as estimated from densitometer scans of the reduced and alkylated subcomponent C1q on 5% polyacrylamide gels run in sodium dodecyl sulphate and the yields of performic acid-oxidized A, B and C chains eluted from DEAE-cellulose, is of the order 1.0:1.0:1.0, which is strong evidence that there are equimolar amounts of each chain in human subcomponent C1q. It is difficult to draw firm conclusions about the subunit and chain structure of human and rabbit subcomponent C1q, from the estimates of molecular weight obtained so far (Table 3). Both human and rabbit subcomponent C1q have a molecular weight of about 410000 and appear to be composed of seven non-covalently linked subunits of approximately 57000 molecular weight. This is consistent with the electron-microscopy studies of subcomponent C1q (Shelton *et al.*, 1972; Svehag *et al.*, 1972), which suggest that there are six to eight non-covalently linked subunits/mol. The physical results given here are, however, probably not sufficiently accurate to favour seven non-covalently linked subunits rather than six or eight. Some of the results, such as the sodium dodecyl sulphate polyacrylamide-gel patterns, the quantitative and qualitative *N*-terminal amino acid analyses and the apparently equimolar yield of each chain after reduction or oxidation, suggest that there are three chains per non-covalently linked subunit. However, the estimates of molecular weight made on the polyacrylamide gels run in sodium dodecyl sulphate and by sedimentation equilibrium studies in guanidinium chloride (Table 3) indicate that there may be two chains per subunit. Sequence analysis of the separated chains should provide the most accurate check on these estimates of molecular weight. The most reliable estimates of molecular weight should be obtained from the gel-filtration and sedimentation-equilibrium studies in 5.3M-guanidinium chloride rather than from the estimates made by using polyacrylamide gels run in sodium dodecyl sulphate. This is because of the influence that carbohydrate and collagen-like portions of the molecule may have on the rate at which subcomponent C1q and the chains derived from it would migrate through polyacrylamide gels in sodium dodecyl sulphate. The presence of carbohydrate has led to the overestimation of molecular weights of immunoglobulin chains (O'Daly & Cebra, 1971; Shubert, 1970); also the chains of collagen and peptides derived from them migrate through polyacrylamide gels in sodium dodecyl sulphate at a much slower rate than globular proteins of the same molecular weight (Furthmayr & Timpl, 1971). It seems possible that the presence of two components in unreduced rabbit subcomponent C1q preparations (Figs. 2*b* and 2*c*) is due to the loss of carbohydrate from one of the chains. This would help to explain the variability in the amount of the lower-molecular-weight component in the different rabbit subcomponent C1q preparations (Figs. 2*b* and 2*c*), since small differences in carbohydrate content would yield different mobilities on the polyacrylamide gels run in sodium dodecyl sulphate. This influence that carbohydrate content of a protein has on its mobility on polyacrylamide gels run in sodium dodecyl sulphate may reconcile some of the differences between the model that Yonemasu & Stroud (1972) have

proposed for human subcomponent C1q and some of the results obtained in our studies. Yonemasu & Stroud (1972) relied almost entirely on molecular-weight estimations made on polyacrylamide gels run in sodium dodecyl sulphate and upon densitometer scans of the protein stained on these gels. They did not consider the effect that carbohydrate might have on their unreduced and reduced preparations of subcomponent C1q. In their model they propose that there are two types of non-covalently linked subunits of molecular weight 60000 and 42000 present in the ratio 6:2. This ratio is very similar to that found in some rabbit subcomponent C1q preparations (Fig. 2*c*). They also propose that the 60000 unit is composed of covalently linked polypeptide chains of molecular weight 29000 and 27000. These would correspond to our A and B chains. They propose that the 42000 unit is composed of a covalently linked dimer of 22000-molecular-weight polypeptide chains, which would correspond to our C chain. We have not been able to demonstrate this sort of relationship, and our results suggest that there is only one type of non-covalently linked subunit in human subcomponent C1q, which on reduction or oxidation yields three chains in equimolar amounts.

The collagenase-digestion experiments (Fig. 4; Table 5) indicate that there are distinct collagen-like regions in the subcomponent C1q molecule, which are important for its haemolytic activity. The amino acid analysis of all three chains of subcomponent C1q is consistent with there being a collagenous region in each (Table 4). The size of the chains (approx. mol.wt. 23000) and the size (6000–10000 mol.wt.) and yield (60%) of the peptides in the non-collagenous residue of the collagenase digest also indicates the presence of regions in each chain, the sequence of which may show homology with collagen chains. Analysis of the soluble portion of the collagenase digest (Table 5) also supports this view, since peptides approaching the expected analysis for collagen of 30% glycine, 22% proline plus hydroxyproline were found in the low-molecular-weight peak from Sephadex G-200 (Table 5). This peak separated on Sephadex G-25 into four main peptide peaks, two of which had the composition 30% glycine, 21% proline plus hydroxyproline and 49% other amino acids, which included relatively high hydroxylysine values and low cystine values, all of which is strong indirect evidence that the repeating sequence of collagen, Gly-X-Y (where X is often proline and Y often hydroxyproline: see Traub & Piez, 1971) is present. The molecular model for human subcomponent C1q, suggested by Shelton *et al.* (1972) from electron-microscope studies, may also be explicable in terms of the peptide chains of subcomponent C1q containing collagen-like regions, i.e. the rod-like 'connecting strands' between the 'terminal' and 'central' globular regions found in their studies. More detailed chemical studies will be necessary to establish the position, and extent, of these collagen-like regions in each of the chains of subcomponent C1q.

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