Purification and characterization of subcomponent C1q of the first component of mouse complement

Kunio YONEMASU and Takako SASAKI

Department of Bacteriology, Nara Medical College, 840 Shijocho, Kashihara, Nara 634, Japan

(Received 8 September 1980/Accepted 18 September 1980)

1. Mouse C1q, a subcomponent of the first component of complement, has been purified in a highly haemolytically active form by a combination of precipitation with EGTA, ion-exchange chromatography and gel filtration. Yields ranged from 3 to 5 mg/200 ml of serum, and the activity of final preparations was in the range 2×10^{13} – 4×10^{13} C1q effective molecules/mg. 2. The molecular weight of mouse C1q was $439\,500 \pm 1586$, as determined by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. 3. Mouse C1q was shown to be composed of non-covalently linked subunits, all being in the molecular-weight range 45 000–46 000, and three covalently linked chains each having a molecular weight of approx. 23 000 as determined on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate by using non-covalently and covalently linked subunits of human C1q as markers with known molecular weights calculated theoretically previously [Porter & Reid (1978) *Nature (London)* **275**, 699–704]. 4. Mouse C1q contained hydroxyproline, hydroxylysine, a high percentage of glycine and approx. 9% carbohydrate. The absorption coefficient and nitrogen content of C1q were also determined.

Complement is a complex mixture of plasma proteins that not only plays an important role in host defence mechanisms but is also implicated in disease processes involving cytotoxic and immune-complex-mediated hypersensitivities (Cooper *et al.*, 1971). Activation of complement can take place either by the classical (reviewed by Porter & Reid, 1979) or alternative pathway (reviewed by Götze & Müller-Eberhard, 1976). C1q is one of the subcomponents of the first component of complement that are present as a Ca^{2+} -dependent macromolecular protein complex in plasma (Lepow *et al.*, 1963) and activates the successive complement components in the classical pathway by interaction of antigen-antibody complexes or aggregated immunoglobulins. Human C1q is a glycoprotein of approx. mol.wt. 400 000 containing hydroxyproline, hydroxylysine and abundant glycine residues (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott & Müller-Eberhard, 1972), and extensive chemical studies have shown that C1q is an unusual globular protein and that about a half of its molecule is composed of triple helix with collagen-like sequences (Reid, 1974, 1976, 1977, 1979). The carbohydrates of human C1q have been studied by several workers (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott &

Müller-Eberhard, 1972) and recent detailed studies have shown that C1q contains hydroxylysine-linked glycosides in collagen-like regions (Shinkai & Yonemasu, 1979) and asparagine-linked sugar chains in C-terminal globular regions (Mizuochi *et al.*, 1978) of its molecule. Thus the overall chemical characteristics of the human C1q molecule are similar to those of procollagen (reviewed by Fessler & Fessler, 1978), type IV (Kefalides, 1975; Levine & Spiro, 1979) or basement-membrane collagens of bovine anterior lens capsule (Schwartz *et al.*, 1980).

It is well known that collagen molecules are widely distributed among the members of the Animal Kingdom, including invertebrates, and that the complement system is probably present in invertebrates and has been conserved throughout evolution (Gigli & Austen, 1971). Not surprisingly, therefore, interest in the biochemistry or structure on C1q of various animals besides man has increased greatly, and purification and partial characterization of rabbit (Reid *et al.*, 1972; Volanakis & Stroud, 1972; Lowe & Reid, 1974), bovine (Campbell *et al.*, 1979), equine (McDonald & Burger, 1979), rat (Höffken *et al.*, 1978) and frog C1q (Alexander & Steiner, 1980) have been reported. Each has been shown to have an unusual amino acid composition (hydroxyproline, hydroxylysine and a high percentage of glycine) and to be composed of

Abbreviations used: SDS, sodium dodecyl sulphate; dansyl, 5-dimethylaminonaphthalene-1-sulphonyl.

essentially the similar subunit structure. Although mice are animals for which immunological mechanisms have been elucidated in most detail, information on their complement system, and especially its biochemistry, is scanty. The present study was therefore undertaken to purify and to characterize mouse C1q. The present paper describes the purification of mouse C1q in a high yield by rapid and simple chromatographic procedures and its chemical and structural characterization.

Materials and methods

Materials

The sources of reagents used were as follows: sheep erythrocytes in Alsever's solution and guinea-pig serum (Microbial Diseases Research Foundation, Osaka University, Osaka, Japan); Sepharose 6B and DEAE-Sephadex A-50 (Pharmacia Fine Chemicals, Tokyo, Japan); CM-cellulose CM-52 (Whatman, Maidstone, Kent, U.K.); agarose (Bio-Rad, Richmond, CA, U.S.A.); polyamide layer sheet (Cheng Chin Trading Co., Taiwan, Republic of China); microtitre plates (Cooke Engineering Co., Alexandria, VA, U.S.A.); EGTA (Frederick Smith Chemical Co., Columbus, OH, U.S.A.); EDTA, polyacrylamide and *NN'*-methylenebisacrylamide (Eastman Organic Chemicals, Rochester, New York, U.S.A.); iodoacetamide, dithiothreitol, bovine liver catalase and standard dansyl-(amino acids) (Sigma Chemical Co., St. Louis, MO, U.S.A.). Urea was purchased from Nakarai Chemicals, Kyoto, Japan, and aqueous solutions of it were absorbed repeatedly with activated charcoal.

Isolation of subcomponent C1q

Human C1q was purified by the method of Yonemasu & Stroud (1971). The similar method of repetitive precipitation with chelating agents could not be used for purification of mouse C1q owing to difficulties encountered in dissolving the precipitate obtained from mouse C1q-rich fraction by dialysis against EDTA, pH 5.0.

Male and female CF-1 mice (2–3 months old) were purchased from Carworth Farms, Portage, MI, U.S.A., and bred in closed colonies in our laboratory. Mouse blood was collected by cutting the carotid artery; it was allowed to clot at 37°C for 60 min and then left at room temperature for 60 min. Serum was separated at 2°C by centrifugation at 2000 *g* for 30 min and re-centrifuged at 20000 *g* for 90 min to remove floating free lipid.

A precipitate from 200 ml of serum was prepared by dialysing the serum successively against 1 litre and 3 litres of 25 mM-EGTA (pH 7.5, conductivity 1.7 mS), for 7 and 13 h at 4°C. The precipitate thus formed (hereafter called the 'EGTA precipitate') centrifuged at 10000 *g* for 30 min, washed

twice with the same EGTA solution, and redissolved in 5 ml of 500 mM-NaCl/10 mM-EDTA/10 mM-borate buffer (pH 8.0, conductivity 45 mS).

After the removal of any insoluble aggregates from the redissolved precipitate by centrifugation at 20000 *g* for 30 min, the clear sample was applied to a column (2.8 cm × 84 cm) of Sepharose 6B equilibrated with the same buffer used for redissolving. The column was eluted at a flow rate of 20 ml/h. The fractions containing C1q were pooled and were concentrated by ultrafiltration in the cold; the conductivity was adjusted to 18 mS by adding water, and the pH was ascertained to be of 8.0. This solution was passed through a column (1.8 cm × 4.8 cm) of DEAE-Sephadex A-50 equilibrated with 200 mM-NaCl/10 mM-EDTA/10 mM-Tris/HCl buffer (pH 8.0, conductivity 18 mS) and eluted with the same buffered solution at a flow rate of 80 ml/h. The eluted fractions containing C1q were pooled and concentrated. After adjusting the conductivity to 8 mS/cm with water and the pH to 6.0 with acetic acid, the solution was applied to a column (1.8 cm × 7.9 cm) of CM-cellulose equilibrated with 50 mM-NaCl/10 mM-EDTA/10 mM-phosphate buffer (pH 6.0, conductivity 8 mS) and eluted with the same buffered solution at a flow rate of 50 ml/h. After the first protein peak was eluted, the column was washed extensively with the same solution until the A_{280} of the eluate became negligible. A linear gradient composed of 300 ml of 50 mM-NaCl/10 mM-EDTA/10 mM-phosphate buffer, pH 6.0, and 300 ml of 300 mM-NaCl/10 mM-EDTA/10 mM-phosphate buffer, pH 6.0, was then applied. The fractions containing C1q were pooled, concentrated, and rechromatographed on the Sepharose 6B under the same conditions used for the second purification step of the redissolved EGTA precipitate. The fractions containing C1q were pooled and concentrated; these represented the finally purified C1q preparations.

Determination of subcomponent C1q through the purification scheme

Because the haemolytic assay of C1q in mouse whole sera or crude preparations was less sensitive and less reproducible, the location of C1q was performed with a single radial immunodiffusion using 1% agarose in 0.1% NaN_3 /150 mM-NaCl/10 mM-EDTA/50 mM-Tris/50 mM-glycine, pH 8.0, containing an appropriate dilution of rabbit anti-(mouse C1q) antiserum. The lower limit of sensitivity of this single radial immunodiffusion was about 3 μg of C1q/ml (corresponding to 30-fold dilution of the original serum). Monospecific rabbit anti-(mouse C1q) antiserum was prepared in advance of purification of C1q by essentially same method as that described previously (Yonemasu, 1975).

Protein determination

Nitrogen determination was performed by H_2SO_4 digestion followed by nesslerization (Chase & Williams, 1968). The percentage of nitrogen and the $A_{1\text{cm}}^{1\%}$ determined at 280nm by using purified C1q after extensive dialysis against water, freeze-drying and drying to a constant weight.

Quantitative amino acid analysis

Samples were hydrolysed in twice-distilled 6M-HCl at 110°C for 24 and 48 h in sealed evacuated tubes. On completion of hydrolysis, the HCl was removed by rotary evaporation at 40°C. The dried hydrolysate was dissolved in 100mM-citric acid, pH2.20, and examined on a Hitachi automatic amino acid analyser (model 835; Hitachi Seisakusho, Tokyo, Japan). Corrections were made for destruction of serine, threonine and tyrosine. Half-cystine was determined as cysteic acid after performic acid oxidation (Hirs, 1956).

N-Terminal analysis

Qualitative N-terminal analyses were performed by using the dansyl method described by Gray (1967, 1972).

Carbohydrate analysis

This was performed as described by Takasaki & Kobata (1978). In order to solubilize C1q completely, Pronase E was added at an enzyme/substrate ratio of 1:10 and allowed to react at 37°C for 24 h. For the determination of neutral and amino sugars, and N-acetylneuraminic acid, these samples were hydrolysed at 100°C in 4M-HCl for 2 and 4 h and in 10mM-HCl for 20 and 30 min respectively. The sum of the separated sugars of mouse C1q was corrected by using the recovery rate of that of human C1q through the entire assay steps from acid hydrolysis to the measurement of radioactivity, which was run in parallel with the former.

Non-covalently linked subunits and covalently linked peptide chains

These were prepared as described previously (Yonemasu & Stroud, 1972).

SDS/polyacrylamide-gel electrophoresis

In order to check the purity of undissociated C1q, a modification of the method of Davis (1964) was used. The gel and electrode buffer contained 0.1% SDS at pH8.3, and samples were run on 4.0% polyacrylamide gels. Analyses of non-covalently linked subunits and covalently linked chains of C1q were performed essentially as described by Fairbanks *et al.* (1971). Samples were analysed on 10% gels.

The gels were scanned at 620 nm by using a Toyo densitometer (model DMU-33C; Toyo Kagakusangyo, Co., Osaka, Japan).

Determination of the molecular weights of subcomponent C1q, its subunits and chains

This was performed by a modification of the method of Dunker & Rueckert (1969). Human C1q, its non-covalently linked subunits (A-B and C-C dimers) and covalently linked chains (A-, B- and C-chains) were used as markers with known molecular weights (410000, 47000 and 44000, 24000, 23000 and 22000 respectively; Porter & Reid, 1978). For determination of the molecular weight of undissociated C1q, human immunoglobulin M, bovine liver catalase and human immunoglobulin G were also used as markers of known molecular weight (900000, 240000 and 160000, respectively).

Haemolytic assay of subcomponent C1q

Sensitized erythrocytes and the stock solution of barbital-buffered saline were prepared as described by Mayer (1961). EAC4 cells were prepared by the method of Borsos & Rapp (1963). All buffered solutions used for C1q haemolytic assay were the same as those described previously (Yonemasu & Stroud, 1971). Functionally pure guinea-pig complement component C2, and human subcomponents C1r and C1s, were prepared as described by Nelson *et al.* (1966) and Assimeh *et al.* (1974) respectively. The late-acting complement components (C3-C9) were supplied in the form of guinea-pig whole serum diluted 1 to 37.5 with 132mM-NaCl/10mM-EDTA/2mM-barbital buffer, pH7.5, containing 0.1% gelatine (w/v).

Serial dilutions (0.25 ml) of the solution to be tested made in 6.5% (w/v) sucrose/44mM-NaCl/15mM- CaCl_2 /1mM- MgCl_2 /2mM-barbital buffer, pH7.4 (conductivity 4.2 mS) containing 0.1% gelatine (w/v), were mixed with an equal volume of EAC4 cells (1.5×10^8 cells/ml) in the same buffer and allowed to react at 30°C for 20 min. After centrifugation at 2000g for 10 min, C1q sites on the EAC1q4 formed were quantified as described previously (Yonemasu & Stroud, 1971).

Immunodiffusion analyses

Ouchterlony double diffusion, immunoelectrophoresis and single radial immunodiffusion were performed by using modifications of the methods of Ouchterlony (1958), Scheidegger (1955) and Mancini *et al.* (1965) respectively. The buffers used for these analyses were same as those described previously (Yonemasu & Stroud, 1971).

Preparation of antiserum to subcomponent C1q

Purified mouse C1q (0.5 mg/ml in 150mM-NaCl)

was mixed with an equal volume of Freund's complete adjuvant and 2.0ml of this mixture were injected into both popliteal and axillar regions subcutaneously. After 3 weeks, each rabbit was given a booster of 100 μ g of C1q subcutaneously into several sites in both shoulder and gluteal regions with 1.0ml of the mixture with Freund's incomplete adjuvant. After another 3 weeks, each rabbit was given an intravenous booster injection of 0.1ml of C1q (1mg/ml in 150mM-NaCl) 3 days successively. The rabbits were bled 10 days after the last booster.

Results

Isolation of subcomponent C1q

C1q was purified from the fresh mouse sera and results of purification are summarized in Table 1. The recovery of C1q in the precipitate with EGTA, which represented approx. 0.7% of the protein in the starting serum based on $A_{280}^{1\text{cm}}$, ranged from 80 to 90%. On a column of Sepharose 6B (Fig. 1), approx. 30% of the protein applied to the column was eluted

in the region of the second, lower and broader, A_{280} peak, which contained 65% of the C1q in the EGTA precipitate. At the third step of purification on DEAE-Sephadex, the material not retained on the column with the starting buffer, representing 70% of the applied protein, contained approx. 44% of C1q in the applied protein. This 'passed-through' material was further purified on CM-cellulose (Fig. 2). C1q was retained on CM-cellulose with the starting buffer and was later eluted by an NaCl gradient over the conductivity range 11–14mS. The pool of the fractions containing C1q was finally rechromatographed on Sepharose 6B.

The final yield of C1q from 200ml of serum, as measured by single radial immunodiffusion, was approx. 3–5mg and represented 14–22% of the starting material.

The purified C1q showed only one single precipitation arc in the slow γ -region against anti-(mouse whole serum) on immunoelectrophoresis. On SDS/polyacrylamide-gel electrophoresis this showed only one protein band (Fig. 3, gel 2), with a slightly slower electrophoretic mobility than that of human

Table 1. Summary of purification of mouse subcomponent C1q
Experimental details are given in the text. C1q amounts were quantified by single radial immunodiffusion.

Purification step	Volume (ml)	$A_{280}^{1\text{cm}}$	$A_{280}^{1\text{cm}} \times \text{volume}$	C1q (mg)	Yield (%)
Serum	200	59.7	11940	21.8	100
Precipitate with EGTA	5	16.1	80.5	18.5	84.8
Sepharose 6B	11	2.25	24.8	12.1	55.5
DEAE-Sephadex	11.6	1.50	17.4	5.3	24.5
CM-cellulose	5.5	1.29	7.1	4.9	22.3
Sepharose 6B	6	0.48	2.9	4.1	18.8

Fig. 1. Chromatography of subcomponent C1q on Sepharose 6B

The C1q fraction obtained by dialysis of 200ml of serum against 25mM-EGTA (pH 7.5, conductivity 1.7mS) was loaded on to a column (2.8cm $\times$ 84cm) of Sepharose 6B equilibrated in 500mM-NaCl/10mM-EDTA/10mM-borate buffer, pH 8.0 (conductivity 45mS). —, A_{280} ; ▨, percentage of C1q compared with that in starting serum.

Fig. 2. Chromatography of subcomponent C1q on CM-cellulose

The C1q fraction from the DEAE-Sephadex column was loaded on to a column (1.8 cm x 7.9 cm) of CM-cellulose equilibrated in 50 mM-NaCl/10 mM-EDTA/10 mM-phosphate buffer, pH 6.0 (conductivity 8 mS). 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 (mS); ▨, Percentage of C1q compared with that in starting serum.

C1q (Fig. 3, gel 1). The molecular weight of mouse C1q was estimated to be approx. 440 000 (average result for seven experiments: $439\,500 \pm 1586$) by using human immunoglobulin M, C1q, immunoglobulin G and bovine liver catalase as markers. From the elution position of mouse C1q on Sepharose 6B with 500 mM-NaCl/10 mM-EDTA/10 mM-borate buffer, pH 8.0 (conductivity 45 mS), the molecular weight of mouse C1q was roughly calculated to be 450 000.

Absorption coefficient and nitrogen content of subcomponent C1q

U.v.-absorption spectra of C1q in 300 mM-NaCl showed a single peak at 278 nm between 251 and 320 nm, and $A_{1\%}^{1\text{cm}}$ at 280 nm was found to be 7.53 ± 0.22 . The percentage of nitrogen in C1q was found to be $14.8 \pm 0.32\%$.

Amino acid and carbohydrate composition of subcomponent C1q

The amino acid compositions of mouse and human C1q are given in Table 2. The overall composition was very similar, and notable features were the high glycine values and the presence of hydroxyproline and hydroxylysine.

The carbohydrate composition of mouse C1q is given in Table 3 in parallel with that of human C1q. Its overall composition was very similar to that of human C1q. The mean total carbohydrate content of mouse C1q determined as the sum of the monosaccharides was 8.71%, and it was slightly higher

Table 2. Amino acid compositions of mouse and human subcomponents C1q

Samples of mouse C1q (0.485 mg) and human C1q (0.643 mg) were hydrolysed for 24 and 48 h. Experimental details are given in the text. Abbreviation used: ND, not determined.

Amino acid	Content (residues/1000 residues)	
	Mouse subcomponent C1q	Human subcomponent C1q
Hyp	37.1	47.6
Asp	84.9	70.1
Thr	58.1	58.9
Ser	68.3	53.6
Glu	88.1	93.2
Pro	59.9	59.8
Gly	170.7	175.7
Ala	42.7	41.4
Cys	17.4	16.5
Val	61.8	54.9
Met	10.4	16.1
Ile	35.2	39.9
Leu	65.3	59.9
Tyr	32.3	36.0
Phe	46.2	45.0
Hyl	18.9	23.0
Lys	38.3	40.0
His	16.5	16.5
Arg	47.9	42.7
Trp	ND	9.1

than that of human C1q. Most of its sugars were in the form of galactose and glucose, and the concentration of galactosamine was slightly lower, but that

Fig. 3. Electrophoresis of purified subcomponent C1q on SDS/4%-polyacrylamide gels

Gel 1, undissociated human C1q; gel 2, undissociated mouse C1q. The anode is to the bottom, and the band near the anode is a non-specific band that forms at the water/buffer interface.

of sialic acid was slightly higher, than that of human C1q.

N-Terminal analysis

Several *N*-terminal analyses of C1q by the dansyl method produced dansyl-threonine and dansyl-aspartic acid.

Non-covalently linked subunits and covalently linked peptide chains of subcomponent C1q

The analyses of these subunits or chains by SDS/polyacrylamide gel electrophoresis are given in comparison with those of human C1q (Fig. 4).

Fig. 4. Electrophoresis of the urea-treated, and reduced and alkylated subunits of subcomponents C1q on SDS/10%-polyacrylamide gels

Gels 1 and 2, non-reduced urea-treated human and mouse C1q respectively; gels 3 and 4, reduced and alkylated human and mouse C1q respectively. The anode is to the bottom.

Under dissociating conditions, the non-reduced mouse C1q gave two intensely stained bands with two faintly stained bands between them (Fig. 4, gel 2), whose electrophoretic mobilities were between those of A-B and C-C dimers of human C1q (Fig. 4, gel 1). The molecular weights of the intensely stained two subunits were estimated to be 46 500 and 45 000 by using non-covalently linked subunits of human C1q as molecular-weight markers. On the densitometer scan, the gel 2 in Fig. 4 gave one scanned area with a molecular weight corresponding to dimers of the major bands, and two areas with a percentage ratio of 14.2:61.8:24.0. The molar ratio of the bands including the 46 500-mol.wt. subunit to the bands including 45 000-mol.wt. subunit was calculated to be 2.49:1. Reduction of mouse C1q gave three stained bands (Fig. 4, gel 4), with almost the same mobilities as

Table 3. *Carbohydrate compositions of mouse and human subcomponent C1q*
Full experimental details are given in the text.

Source of C1q ...	Carbohydrate content			
	Mouse		Human	
	Percentage by weight	Residues/molecule	Percentage by weight	Residues/molecule
Fucose	0.26	6.9	0.21	5.2
Mannose	0.80	19.6	0.66	15.0
Galactose	2.86	69.8	2.88	65.5
Glucose	2.66	64.9	2.52	57.3
Glucosamine	1.35	33.1	1.06	24.3
Galactosamine	0.25	6.2	0.27	6.2
Sialic acid*	0.53	7.5	0.38	5.0
Total	8.71	208	7.98	178.5

* Calculated as *N*-acetylneuraminic acid.

those of A-, B- and C-chains of human C1q (Fig. 4, gel 3). The molecular weights of these three peptide chains were estimated to be 24 000, 23 250 and 22 500 by using A-, B- and C-chains of human C1q as markers. On the densitometer scan, the gel 4 in Fig. 4 gave one scanned area with molecular weight corresponding to dimers of the major bands and three areas with a percentage ratio of 16.2:26.7:30.8:26.3. The molar ratio of these three peptide chains was found to be 1.00:1.20:1.06. The average molar ratio in three experiments on different preparations was found to be 1.00:1.18:1.06, which approximates to 1:1:1.

Quantitative titration of subcomponent C1q haemolytic activity

The haemolytic activity of purified C1q was quantified. A purified preparation of C1q gave 2.46×10^{13} effective molecules/mg.

Immunodiffusion

This was done in Ouchterlony double diffusion done in agarose gels. Mouse C1q gave a single line against rabbit anti-(mouse C1q) antiserum, and also against rabbit anti-(mouse whole serum) antiserum. Rabbit anti-(mouse C1q) antiserum gave a single line against mouse whole serum as well as C1q. These precipitin lines were abolished by heating the mouse serum or C1q at 56°C for 30 min beforehand.

Discussion

Highly purified and haemolytically active C1q samples from various animals besides man are increasingly important for studies on structural similarities and the possible interchangeability of biological activities among these subcomponents

throughout evolution. In the present paper we have shown that a higher yield of haemolytically active mouse C1q has been possible by using an EGTA precipitate than by using euglobulin as a starting material. Since the non-C1q proteins in the EGTA precipitate are much less than those in euglobulin, the column size and time required for purification of C1q by this method appear to be much less (and thus more economical) than by the method using euglobulin as a starting material. The reason why assay of C1q through the purification steps has to be achieved by its antigenicity on single radial immunodiffusion is that the haemolytic assay of C1q in mouse whole sera or crude preparations is less sensitive and less reproducible, possibly owing to the presence of some inhibitory substance(s) in these samples similar to that reported in human serum (Conradie *et al.*, 1975). Consequently, the presentation of the recovery rate of some complement components through their purification (e.g. human C1q) with only their biological activities (Kolb *et al.*, 1979) does not seem to be meaningful. Starting with 200 ml of serum it has been possible to isolate 3–5 mg of C1q (Table 1).

The overall amino acid composition of mouse C1q is very similar to that of human C1q (Table 2) and those reported for the rabbit (Reid *et al.*, 1972) and bovine C1q (Campbell *et al.*, 1979). Notable features of the amino acid composition are the high glycine content and the presence of hydroxyproline and hydroxylysine, suggestive of a collagen-like sequence in mouse C1q also. Analyses of the carbohydrate composition of mouse C1q revealed the presence of galactose and glucose as major components, this also suggesting the possible existence of hydroxylysine-linked disaccharides in its molecule, similar to the situation in human C1q (Shinkai & Yonemasu, 1979) as well as in collagen

molecules. The presence of glucosamine and the significantly higher sialic acid content in the former (Table 3) may suggest the existence of somewhat different asparagine-linked sugar chains from those in the latter (Mizuochi *et al.*, 1978).

The molecular weight of undissociated mouse C1q has been estimated to be approx. 440 000 by SDS/polyacrylamide-gel electrophoresis. This value agreed well with the mol.wt. of 450 000 based on the elution position of C1q on Sepharose 6B.

When the haemolytic activity of highly purified C1q was assayed by using EAC4 cells, functionally purified human C1r and C1s, guinea pig C2 and late-acting components C3–C9, the effective molecules/mg of C1q were found to be 2.46×10^{13} . In this assay system, human C1q was also haemolytically active (Yonemasu & Stroud, 1971). Thus it was found that mouse and human C1q are interchangeable in the formation of haemolytically active EAC1q4, which can activate human C1r and/or C1s successively.

The subunit composition of mouse C1q was analysed by SDS/polyacrylamide-gel electrophoresis. The results obtained are consistent with mouse C1q being composed of 18 polypeptide chains after reduction and alkylation; these chains may be present as disulphide-linked dimers before reduction and alkylation (Fig. 4, gel 2 and 4), but the presence of two major and two minor staining bands in the dissociated but non-reduced sample (Fig. 4, gel 2) makes a full interpretation of the results difficult. At present, we do not know why non-covalently linked subunits of mouse C1q migrate in four bands on SDS/polyacrylamide-gel electrophoresis instead of migrating in two bands as do those of human C1q. Possibly though, these heterogeneous migrations may be caused by polymorphism due to the use of CF-1 strain of mouse instead of an inbred one, or by more differences of sugar chain structures on non-covalently linked subunits in mouse C1q than in human C1q (Mizuochi *et al.*, 1978; Shinkai & Yonemasu, 1979), which become less obvious on the lower-molecular-weight peptide chains after extensive reduction and alkylation. Although additional experiments are required to clarify these points, it seems likely that mouse C1q has a subunit structure similar to that of human C1q.

We thank Dr. K. Kondo, Department of Immunology, Research Institute for Microbial Diseases, University of Osaka, for his instruction in how to perform N-terminal analyses by the dansyl method. We also thank staff in the First Department of Biochemistry, Kobe University School of Medicine, for their instruction in how to perform carbohydrate analyses by Takasaki & Kobata's (1978) microdetermination method. This study was supported in part by The Naito Research Grant for 1979 and The Daiwa Health Research Grant for 1979.

References

- Alexander, R. J. & Steiner, L. A. (1980) *J. Immunol.* **124**, 1418–1425
- Assimeh, S. N., Bing, D. H. & Painter, R. H. (1974) *J. Immunol.* **113**, 225–234
- Borsos, T. & Rapp, H. J. (1963) *J. Immunol.* **91**, 851–858
- Calcott, M. A. & Müller-Eberhard, H. J. (1972) *Biochemistry* **11**, 3443–3450
- Campbell, R. D., Booth, N. A. & Fothergill, J. E. (1979) *Biochem. J.* **177**, 531–540
- Chase, M. W. & Williams, C. A. (1968) *Methods Immunol. Immunochem.* **2**, 266–270
- Conradie, J. D., Volanakis, J. E. & Stroud, R. M. (1975) *Immunochemistry* **12**, 967–971
- Cooper, N. R., Polley, M. J. & Müller-Eberhard, H. J. (1971) in *Immunological Diseases* (Samter, M., ed.), pp. 289–302, Little, Brown and Co., Boston, MA
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
- Dunker, A. K. & Rueckert, R. R. (1969) *J. Biol. Chem.* **244**, 5074–5080
- Fairbanks, G., Stech, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Fessler, J. H. & Fessler, L. I. (1978) *Annu. Rev. Biochem.* **47**, 129–162
- Gigli, I. & Austen, K. F. (1971) *Annu. Rev. Microbiol.* **25**, 309–332
- Götze, O. & Müller-Eberhard, H. J. (1976) *Adv. Immunol.* **24**, 1–35
- Gray, W. R. (1967) *Methods Enzymol.* **11**, 139–151
- Gray, W. R. (1972) *Methods Enzymol.* **25**, 129–136
- Hirs, C. H. W. (1956) *J. Biol. Chem.* **219**, 611–621
- Höffken, K., McLaughlin, P. J., Price, M. R., Preston, V. E. & Baldwin, R. W. (1978) *Immunochemistry* **15**, 409–412
- Kefalides, N. A. (1975) *Dermatologica* **150**, 4–15
- Kolb, W. P., Kolb, L. M. & Podack, E. R. (1979) *J. Immunol.* **122**, 2103–2111
- Lepow, I. H., Naff, G. B., Todd, E. W., Pensky, T. & Hinz, C. F., Jr. (1963) *J. Exp. Med.* **117**, 983–1008
- Levine, M. J. & Spiro, R. G. (1979) *J. Biol. Chem.* **254**, 8121–8124
- Lowe, D. M. & Reid, K. B. M. (1974) *Biochem. J.* **143**, 256–272
- 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
- McDonald, T. L. & Burger, D. (1979) *Immunology* **37**, 517–527
- Mizuochi, T., Yonemasu, K., Yamashita, K. & Kobata, A. (1978) *J. Biol. Chem.* **253**, 7404–7409
- Nelson, R. A., Jensen, J., Gigli, I. & Tamura, N. (1966) *Immunochemistry* **3**, 111–135
- Ouchterlony, Ö. (1958) *Prog. Allergy* **5**, 1–78
- Porter, R. R. & Reid, K. B. M. (1978) *Nature (London)* **275**, 699–704
- Porter, R. R. & Reid, K. B. M. (1979) *Adv. Protein Chem.* **33**, 1–71
- 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. (1979) *Biochem. J.* **179**, 367–371
- Reid, K. B. M., Lowe, D. M. & Porter, R. R. (1972) *Biochem. J.* **130**, 749–763
- Scheidegger, J. J. (1955) *Int. Arch. Allergy* **7**, 103–110
- Schwartz, D., Chin-Quee, T. & Veis, A. (1980) *Eur. J. Biochem.* **103**, 21–27
- Shinkai, H. & Yonemasu, K. (1979) *Biochem. J.* **177**, 847–852
- Takasaki, S. & Kobata, A. (1978) *Methods Enzymol.* **50**, 50–54
- Volanakis, J. E. & Stroud, R. M. (1972) *J. Immunol. Methods* **2**, 25–34
- Yonemasu, K. (1975) *J. Immunol. Methods* **9**, 185–194
- Yonesamu, K. & Stroud, R. M. (1971) *J. Immunol.* **106**, 304–313
- Yonemasu, K. & Stroud, R. M. (1972) *Immunochemistry* **9**, 545–554
- Yonemasu, K., Stroud, R. M., Niedermeier, W. & Butler, W. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 1388–1394