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# Hydroxylysine-Linked Glycosides of Human Complement Subcomponent C1q and of Various Collagens

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1. Human C1q, a subcomponent of the first component of complement, contains 67 disaccharides (glucosylgalactose) and 2.4 monosaccharides (galactose) linked to hydroxylysine in one molecule. It was found that 82.6% of the hydroxylysine residues were glycosylated. The suggestion of the possible existence of glucosylgalactosylhydroxylysine reported previously [Yonemasu, Stroud, Niedermeir & Butler (1971) *Biochem. Biophys. Res. Commun.* **43**, 1388–1394] was confirmed. 2. The hydroxylysine-glycosides are not detected in the *C*-terminal, non-collagen-like, globular regions, but only in the collagen-like regions in the subcomponent C1q molecule. 3. α1(I) and α2 in pig skin, α1(II) in bovine cartilage and α1(III) in bovine skin collagens contain 2.0, 2.2, 13.2 and 2.0 residues of hydroxylysine-glycosides per molecule, respectively. The percentage of hydroxylysine residues glycosylated in each of these chains is relatively low (on average 38%). 4. Neither the high percentage of hydroxylysine residues glycosylated nor the high values for the ratios of disaccharides to monosaccharides in the subcomponent C1q resembles that in α1(I), α2, α1(II) and α1(III). 5. Similarities between the extent of glycosylation of hydroxylysine residues in collagen-like regions in the subcomponent C1q molecule and that of the collagenous constituents of human glomerular basement membranes, aortic intima, skin A- and B-chains and of bovine anterior lens capsule are discussed.

Activation of the complement system in the classical pathway starts by interaction of antigen–antibody complexes or aggregated immunoglobulins with subcomponent C1q of the first component of complement. Subcomponent C1q is a glycoprotein of approx. mol.wt. of 400000, containing hydroxyproline, hydroxylysine and abundant glycine residues (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott & Müller-Eberhard, 1972). Its haemolytic activity is easily destroyed on digestion by bacterial collagenase (Reid *et al.*, 1972), and recent extensive chemical studies have shown that subcomponent 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; Reid & Porter, 1976). The carbohydrate composition of subcomponent C1q was analysed by several workers (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott & Müller-Eberhard, 1972), and the results indicated that glucose and galactose were major components, with smaller amounts of glucosamine, mannose, fucose and sialic acid. Although the possibility of the presence of hydroxylysine-linked glycosides similar to collagens in the subcomponent C1q molecule, on the basis of the poor susceptibility of hydroxylysine to periodate oxidation, has been reported previously (Yonemasu *et al.*, 1971), no direct evidence has been given. Therefore this study was undertaken to identify hydroxylysine-linked glycosides and the location of these sugar chains in the subcomponent C1q molecule.

The hydroxylysine-linked sugars of type-I, -II and -III collagens were also analysed and compared with those of subcomponent C1q. The extent of glycosylation of hydrosylysine residues and the ratio of disaccharides to monosaccharides in subcomponent C1q were also compared with those reported for collagenous constituents of human glomerular basement membranes (Kefalides, 1975), human aortic intima and skin A- and B-chains (Chung *et al.*, 1976) and of bovine anterior lens capsule (Dixit, 1978).

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## Materials and Methods

### *Preparation of human subcomponent C1q*

Human subcomponent C1q was purified from fresh serum by repeated precipitation in EGTA and EDTA solutions of low ionic strengths as previously described (Yonemasu & Stroud, 1971). For sugar analyses, subcomponent C1q was extensively dialysed against water in the cold and then freeze-dried.

*C-Terminal non-collagen-like (globular) region and collagen-like region of subcomponent C1q*

Purified subcomponent C1q (5.8mg) was digested with 0.29mg of collagenase (Seikagaku Kogyo Co., Tokyo, Japan; further purified on hydroxyapatite to remove any caseinolytic activity as described by Matsubayashi *et al.*, 1977) or with 0.20mg of pepsin (Sigma Chemical Co., St. Louis, MO, U.S.A.; 2× crystallized) by methods of Reid *et al.* (1972) and Reid (1976) respectively. The digest was centrifuged at 60000*g* for 30min at 2°C and the resulting precipitate was washed with 3×1ml of each digestion buffer and then with 3×1ml of water. Finally, each precipitate was redissolved in 0.5ml of 0.5M-$NH_4HCO_3$ and then freeze-dried. These two preparations were taken as being derived from the *C*-terminal, non-collagen-like, globular regions (Reid, 1974, 1976) of subcomponent C1q. The supernatant of the pepsin digest was further fractionated on Sephadex G-200 as described by Reid (1976), and the first peak obtained by monitoring the $A_{230}$ was pooled as collagen-like region (fraction P1) (Reid, 1976). This collagen-like region also contains the *N*-terminal non-collagen-like region and several amino acid residues from the globular region (Reid, 1976, 1977). This preparation was freeze-dried after extensive dialysis against cold water.

*Preparation of α1(I) and α2 of pig skin collagen*

These collagens were purified from pig skin by the method of Shinkai *et al.* (1974). In brief, 30ml of acid-soluble collagen (2.3mg/ml in 0.06M-sodium acetate buffer, pH4.8) was centrifuged at 100000*g* for 1h and the resulting supernatant was denatured at 50°C for 20min. This was fractionated into α1+β11 and β12+α2 fractions on a column (1.5cm×10cm) of CM-cellulose at 45°C as described by Piez *et al.* (1963). To remove β12 from β12+α2 fraction, a Sepharose 6B column (2.6cm×120cm), equilibrated and eluted with 2.0M-$CaCl_2$ in 0.05M-Tris/HCl buffer, pH7.5, was used.

*Preparation of α1(II) of bovine cartilage*

Finely sliced costal cartilage was homogenized in 0.5M-sodium acetate with an Ultra-Turrax instrument (KG; Janke und Kunkel, Staufen, Germany) and then stirred at 4°C for 2 days. The insoluble residues were collected by centrifugation at 100000*g* for 30min and dissolved in 0.5M-acetic acid. This was digested by pepsin as described by Miller (1972). The pepsin-solubilized collagen was precipitated by the addition of NaCl to a concentration of 0.9M. The precipitates were redissolved in 1.0M-NaCl in 0.05M-Tris/HCl buffer, pH7.5, and then further fractionated by the use of fractional precipitation similar to the technique described by Trelstad *et al.* (1972), by using successive dialyses against 1.5M-, 2.4M- and 3.6M-NaCl in 0.05M-Tris/HCl buffer, pH7.5. Type-II collagen was recovered in the precipitate obtained at the last step with 3.6M-NaCl in 0.05M-Tris/HCl buffer, pH7.5.

*Preparation of α1(III) of bovine skin*

Type-III collagen was obtained from bovine skin by the method of Chung & Miller (1974). The second major peak of $A_{230}$ from the CM-32 column was further purified by Sepharose 6B under the same conditions used for preparing α2.

*Preparation of hydroxylysine-disaccharides and identification of them with hydroxylysine-galactosylglucose*

Hydroxylysine-disaccharides were prepared by using a modification of the methods described by Spiro (1972). About 40g of the sea sponge *Spongia graminea* was hydrolysed in a sealed tube with 2.0M-NaOH at 105°C for 24h under reduced pressure. After adjustment of the hydrolysate to pH3.0 with 1M-acetic acid, the mixture was centrifuged at 12000*g* for 30min. The resulting supernatant was applied to a column (3.4cm×20cm) of Dowex 50-X8 ($H^+$ form), and the column was washed successively with 450ml of 1mM-acetic acid, 800ml of 8% (v/v) pyridine and 600ml of 3M-$NH_3$. The eluate with 3M-$NH_3$ was concentrated to 15ml by evaporation, 5ml of which was then chromatographed on Bio-Gel P-2 (100–200mesh; 2.4cm×100cm) equilibrated with pyridine/formic acid/water (0.85:1.0:98, by vol.) buffer, pH3.5. The neutral sugars and amino acids were assayed by the phenol/$H_2SO_4$ method (Dubois *et al.*, 1956) and by the ninhydrin reagent respectively. The fraction which contained both amino acids and neutral sugars, and whose molecular weight corresponded to that of hydroxylysine-galactosylglucose, was eluted between elution volumes 260–300ml from the column. This fraction was evaporated to dryness and then dissolved in a small amount of water. A preparative high-voltage paper electrophoresis was performed on Whatman no. 1 paper (60cm×60cm), with pyridine/formic acid/water (0.85:1.0:98, by vol.) buffer, pH3.5, at 30V/cm for 1.5h in parallel with authentic hydroxylysine. The part with a relative mobility of 0.58 to the standard hydroxylysine was extracted with water and further purified by rechromatography on the Bio-Gel P-2 column.

To identify this preparation with hydroxylysine-glycosides, 1mg of the preparation was hydrolysed in a sealed tube with 1ml of 6M-HCl at 100°C for 24h under $N_2$. The hydrolysate was evaporated to dryness and redissolved in 50μl of water. A sample was then analysed by high-voltage paper electrophoresis in parallel with the untreated preparation as well as the authentic hydroxylysine. Amino acids were stained with ninhydrin reagent and the stained areas were scanned spectrophotometrically at 570nm

by using a Chromatoscanner (Shimadzu model CS 910; Shimadzu Seisakusho, Kyoto, Japan). The relative mobility of the stained area of the hydrolysate coincided completely with that of the authentic hydroxylysine and no other amino acids were detected. On the other hand, the untreated preparation moved with a relative mobility of 0.58 to the authentic hydroxylysine.

Neutral sugars of these hydroxylysine-glycosides were also analysed by g.l.c. by using a modification of the method described by Porter (1975). In brief, 0.9mg of hydroxylysine-glycosides was hydrolysed with 400$\mu$l of 0.01M-HCl containing 20% (w/v) Dowex 50W ($H^+$ form) at 100°C for 48h. The hydrolysates were deaminated with $NaNO_2$ for 30min at room temperature and the excess $Na^+$ was removed by adding 100$\mu$l of 40% (w/v) Dowex 50W ($H^+$ form), to convert all the $NaNO_2$ into $HNO_2$. After addition of 1$\mu$mol of *myo*-inositol as an internal standard, the reaction mixtures were applied to a combined column of Dowex 50W ($H^+$ form) (0.6cm×2cm) and of Dowex 1-X2 ($HCO_3^-$ form) (0.6cm×2cm). The column was washed successively with water (2ml) and 50% (v/v) methanol (2ml). The eluates were pooled and evaporated to dryness. The residues were then dissolved in 100$\mu$l of water, and an equal volume of 0.22M-$NaBH_4$ was added. To the resulting reaction mixture, a small volume of acetic acid was added and evaporated to decompose the excess $NaBH_4$. Borate was removed as volatile trimethyl borate by adding 1ml of methanol/HCl (1000:1, v/v) four times under reduced pressure. The alditol was acetylated with 0.5ml of acetic anhydride and an equal volume of pyridine at 100°C for 1h. After evaporation of the solvents with toluene, the resulting alditol acetate was dissolved in 100$\mu$l of chloroform and then assayed with a gas chromatograph (model 400; F and M Scientific Co., Avondale, PA, U.S.A.) at a constant temperature of 190°C by using a U column (5mm × 200cm) of 3% ECNSS-M-coated Gas-Chrom Q (80–100mesh). By analysis in four experiments, approximately equimolar amounts of galactose and glucose were found and no other sugars were detected in this preparation. The amount of these sugars was also approximately equal on a molar basis to that of hydroxylysine quantified with authentic hydroxylysine by using the Chromatoscanner.

For the assay of hydroxylysine-glycosides in subcomponent C1q and in chains of various collagens, this preparation of hydroxylysine-galactosylglucose was used as an internal standard.

*Preparation of hydroxylysine-galactose*

This was carried out by using purified hydroxylysine-galactosylglucose by the method of Spiro (1967). The preparation of hydroxylysine-galactose moved with a relative mobility of 0.64 compared with standard authentic hydroxylysine on the high-voltage paper electrophoresis.

*Preparation of samples for assay and quantification of hydroxylysine-glycosides*

The measured amounts (2–5mg) of freeze-dried and desiccated subcomponent C1q, $\alpha$1(I) and $\alpha$2 from pig skin collagen, $\alpha$1(II) from bovine cartilage and $\alpha$1(III) from bovine skin were hydrolysed with 1.0ml of 2M-NaOH at 105°C for 24h in sealed evacuated tubes. The hydroxylysine and its glycosides were adsorbed on a column (0.6cm×2cm) of Dowex 50W ($H^+$ form) and eluted with aq. $NH_3$, as described for the preparation of hydroxylysine-disaccharides. The eluates were evaporated to dryness and the residues were dissolved in 50$\mu$l of water. Portions of each sample (usually 1–10$\mu$l) were analysed by high-voltage paper electrophoresis in parallel with various amounts of authentic hydroxylysine as well as its glycosides. Amino acids were stained with ninhydrin reagent and were directly measured spectrophotometrically at 570nm by using a Shimadzu Chromatoscanner. That the amounts of authentic hydroxylysine (2–10nmol) applied on paper and the relative intensity of colorimetric areas gave a straight-line correlation by this method was confirmed. The sensitivity of this method was about 1nmol of hydroxylysine residue. Since the recovery rate of hydroxylysine residues with galactosylglucose units from standard purified hydroxylysine-disaccharides through the entire steps from alkaline hydrolysis to spectrophotometrical assay in three experiments was 82±1.7% (mean ±S.E.M.), the values obtained from test samples were corrected by using a factor of 0.82. Although the sum of free and glycoside-coupled hydroxylysine residues agreed well with those obtained by amino acid analyses, total hydroxylysine residues of each sample were mainly calculated from the quantity of protein used for the assay and the number of hydroxylysine residues examined or reported previously [Reid (1974, 1977; Reid & Thompson, 1978) for subcomponent C1q; Strawich & Nimni (1971) for $\alpha$1(II); Fujii & Kühn (1975) for $\alpha$1(III)] by amino acid analyses.

To locate the hydroxylysine-glycosides in the subcomponent C1q molecule, precipitates obtained by collagenase or pepsin digestion and fraction P1 (collagen-like region from the supernatant after pepsin digestion) were hydrolysed and assayed similarly.

*Amino acid analysis of $\alpha$1(I) and $\alpha$2 of pig skin collagen*

Samples were hydrolysed in twice-distilled 6M-HCl at 110°C for 24h in sealed evacuated tubes, and amino acid analyses were performed on an automatic amino acid analyser (Jero 6SH; Nihondenshi, Tokyo, Japan).

## Results and Discussion

*Quantitative analysis of hydroxylysine-glycosides of human subcomponent C1q, α1(I) and α2 of pig skin, α1(II) of bovine cartilage and α1(III) of bovine skin*

The amounts of hydroxylysine-linked glycosides in subcomponent C1q were determined and are shown in Table 1. Subcomponent C1q contained 67 glucosylgalactose groupings and 2.4 galactose residues linked to hydroxylysine in one molecule. The percentage of hydroxylysine residues glycosylated to those calculated from amino acid-sequence studies of Reid (1974, 1977; Reid & Thompson, 1978) was 82.6% and almost all of the glycosides (96.5%) were disaccharides. These results are direct evidence for the presence of hydrosylysine-glycosides in the human subcomponent C1q molecule, and has confirmed the suggestive data reported previously by several workers (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott & Müller-Eberhard, 1972). That the extent of glycosylation of hydroxylysine residues in the subcomponent C1q molecule was high (82.6%) was not contradictory to the poor susceptibility of hydroxylysine residues in subcomponent C1q to periodate oxidation reported previously (Yonemasu *et al.*, 1971).

Some discrepancy in the number of hydroxylysine residues (84 residues) calculated by Reid (1974, 1977) and Reid & Thompson (1978) on the basis of amino acid-sequence studies and that from the sum of free and glycoside-coupled hydroxylysine residues (74 residues) was found. This discrepancy may be caused by a lower recovery rate of hydroxylysine disaccharides [through the entire steps from alkaline hydrolysis, passage through a column of Dowex 50W ($H^+$ form) and spectrophotometric assay] derived from subcomponent C1q compared with that for the standard hydroxylysine-disaccharides. Since the sum of free and glycoside-coupled hydroxylysine residues, calculated by using a factor of 0.82 as the recovery rate for each chain of collagens, agreed well with the number of hydroxylysine residues obtained from amino acid analysis (Table 1), the reason for the possible lower recovery rate with subcomponent C1q appeared to be due to its structural and/or physicochemical complexity.

Purified α1(I) and α2 of pig skin, α1(II) of bovine cartilage and α1(III) of bovine skin contained 1.2, 0.9, 10.4 and 1.4 residues of hydroxylysine-galactosylglucose and 0.8, 1.3, 2.8 and 0.6 residues of hydroxylysine-galactose in each molecule of the chains respectively (Table 1). The values of hydroxylysine-glycosides in these interstitial collagens were not integers, which appeared to be the result of molecular micro-heterogeneity. Ratios of hydroxylysine residues with glycosides (galactosylglucose or galactose) to total hydroxylysine residues in each chain of these interstitial collagens were calculated to be 40, 24.4, 47.1 and 40% for α1(I), α2, α1(II) and α1(III) respectively. As shown in Table 1, the ratios of hydroxylysine-disaccharides to hydroxylysine-glycoside residues in each chain of α1(I), α2, α1(II) and α1(III) were calculated to be 60, 40.9, 78.8 and 70% respectively. The extent of glycosylation of hydroxylysine residues in each chain of these interstitial collagens was much lower than that of subcomponent C1q. The ratio of hydroxylysine residues coupled with disaccharide to hydroxylysine-glycoside residues in the former was also low in comparison with that for subcomponent C1q.

Table 1. *Hydroxylysine and hydroxylysine-linked glycosides of human complement subcomponent C1q, pig skin type-I and bovine cartilage type-II and skin type-III collagens*

Full experimental details are given in the text. The results are expressed as the number of residues/molecule of human complement subcomponent C1q or chain of collagens. Values in parentheses are percentages calculated from the experimental values.

| | Human subcomponent C1q | Pig skin | | Bovine cartilage | Bovine skin |
|---|---|---|---|---|---|
| | | α1(I) | α2 | α1(II) | α1(III) |
| Hydroxylysine | 84* | 5 | 9 | 28† | 5‡ |
| Hydroxylysine free of glycoside | 4.5 | 2.5 | 6.5 | 13.5 | 2.7 |
| Hydroxylysine-glycosides | 69.4 | 2.0 | 2.2 | 13.2 | 2.0 |
| (% glycosylation to hydroxylysine)§ | (82.6) | (40) | (24.4) | (47.1) | (40) |
| Hydroxylysine-linked galactosylglucose | 67 | 1.2 | 0.9 | 10.4 | 1.4 |
| Hydroxylysine-linked galactose | 2.4 | 0.8 | 1.3 | 2.8 | 0.6 |
| (% disaccharide to total hydroxylysine-glycosides) | (96.5) | (60) | (40.9) | (78.8) | (70) |

* Hydroxylysine residues calculated from Reid (1974, 1977; Reid & Thompson, 1978).

† Hydroxylysine residues calculated from Strawich & Nimni (1971).

‡ Hydroxylysine residues calculated from Fujii & Kühn (1975).

§ Values are percentages of hydroxylysine residues glycosylated to total hydroxylysine residues calculated from amino acid analyses.

*Location of hydroxylysine-glycosides in the subcomponent C1q molecule*

To locate the hydroxylysine-glycosides in the subcomponent C1q molecule, the precipitate obtained by prolonged collagenase digestion or by limited pepsin digestion was used for assay of the *C*-terminal, non-collagen-like, globular region of the subcomponent C1q molecule. The supernatant of the pepsin digest was further fractionated on Sephadex G-200, and the first $A_{230}$ peak was pooled as fraction P1 and used as the entire collagen-like region together with the *N*-terminal non-collagen-like region of subcomponent C1q (Reid, 1976). A portion (one-tenth of the total) of alkaline hydrolysate of 2.6mg of precipitate obtained by collagenase digestion or of 0.58mg of that obtained by pepsin digestion was analysed for their content of hydroxylysine-glycosides. Neither the precipitate (0.26mg) obtained by collagenase digestion nor that (0.058mg) obtained by pepsin digestion contained any detectable hydroxylysine-galactosylglucose and/or hydroxylysine-galactose. On the other hand, considerable amounts of hydroxylysine-disaccharides and trace amounts of its monosaccharides were detected in 0.18mg of fraction P1 (collagenous region) of the subcomponent C1q molecule. The amount of precipitate obtained by collagenase digestion (0.26mg), that obtained by pepsin digestion (0.058mg), and the amount of the collagenous fraction P1 (0.18mg) were estimated to be derived from approx. 1.3nmol, 0.29nmol and 0.9nmol of subcomponent C1q based on the polypeptide-chain model of human subcomponent C1q proposed by Reid & Porter (1976). Considering the sensitivity of our assay method, the amount of precipitate of collagenase digest, which corresponded to the *C*-terminal, non-collagen-like, globular region of 1.3nmol of subcomponent C1q, was sufficient for one hydroxylysine residue to be detected. Since the *N*-terminal, non-collagen-like, region of subcomponent C1q does not contain hydroxylysine residues (Reid, 1974, 1976; Reid & Thompson, 1978), the detection of hydroxylysine-glycosides in the collagenous fraction P1, but not in either precipitate, indicates that all of the hydroxylysine-linked glycosides appear to be exclusively located in the collagen-like regions of the subcomponent C1q molecule.

The carbohydrate composition of human subcomponent C1q reported by several workers (Yonemasu *et al.*, 1971; Reid *et al.*, 1972; Calcott & Müller-Eberhard, 1972) has also indicated the presence of smaller amounts of glucosamine, mannose, fucose and sialic acid, as well as glucose and galactose, the major sugar components. A carbohydrate analysis of human subcomponent C1q has shown that it also contains asparagine-linked sugar chains, which are composed of the so-called serum-type sugars (galactose, glucosamine, mannose, fucose and sialic acid) (Mizuochi *et al.*, 1978). Interestingly, the asparagine-linked sugar chains are located exclusively in the *C*-terminal, non-collagen-like, globular regions of the subcomponent C1q molecule. The different location of hydroxylysine-linked sugar chains and of asparagine-linked sugar chains in the subcomponent C1q molecule suggests that these sugar chains may be of importance when the subcomponent C1q molecule fulfils its biological activities.

Table 2. *Hydroxylysine-glycosides and some amino acids from the collagenous region of human complement subcomponent C1q and collagenous constituents of human glomerulus, aortic intima and skin A- and B-chains and of bovine anterior lens capsule*

The values are expressed as the number of residues/1000 residues. Values in parentheses are percentages calculated from these values.

| | Human subcomponent C1q (fraction P1) | Human glomerulus† | Human aortic intima† | Human skin‡ | | Bovine anterior lens capsule§ | |
|---|---|---|---|---|---|---|---|
| | | | | A-chain | B-chain | 110000 mol.wt. | 50000 mol.wt. |
| Hydroxylysine | 47* | 44.6 | 48 | 22 | 39 | 64 | 45 |
| (% glycosylation to hydroxylysine) | (82.6) | (80.7) | (93.8) | (36.3) | (87.2) | (90.6) | (75.6) |
| (% disaccharide to hydroxylysine-glycoside) | (96.5) | (94.4) | (91.1) | (62.5) | (85.3) | (89.7) | (82.4) |
| Half-cysteine | 8.9 | 8.0 | 20 | 0 | 0 | 2.9 | 1.8 |

* Amino acid residues calculated from Reid (1974, 1976, 1977; Reid & Thompson, 1978) as fraction P1.
† Composition calculated from Kefalides (1975).
‡ Composition from Chung *et al.* (1976).
§ Composition from Dixit (1978).

*Comparison of hydroxylysine-linked glycosides of subcomponent C1q with those of collagenous constituents of type-IV and so-called membrane-type collagens*

Since neither the extent of glycosylation of hydroxylysine residues nor the ratio of disaccharides to monosaccharides in hydroxylysine-glycosides in the subcomponent C1q molecule resembles those in chains of interstitial collagens (Table 1), comparison was made of hydroxylysine-linked glycosides of subcomponent C1q with those of collagenous constituents of the human glomerulus (Kefalides, 1975), human aortic intima and skin A- and B-chains (Chung *et al.*, 1976), and of bovine anterior lens capsule (Dixit, 1978). Amino acid residues of subcomponent C1q were calculated only from those of the collagenous regions (fraction P1; Reid, 1974, 1977; Reid & Thompson, 1978) and are shown in Table 2. Except for the much lower extent of glycosylation of hydroxylysine residues of collagenous constituents in human skin A-chain (36.3%), those in human glomerulus (80.7%), aortic intima (93.8%), skin B-chain (87.2%) and both collagenous constituents in bovine anterior lens capsule (90.6% for the constituent with the higher molecular weight and 75.6% for that with the lower molecular weight) are comparable with that of subcomponent C1q (82.6%). The amounts of disaccharide expressed as a percentage of the total hydroxylysine-glycoside residues of collagenous components of human glomerulus (94.4%), aortic intima (91.1%), skin B-chain (85.3%) and of bovine anterior lens capsule with the higher molecular weight (89.7%) are also comparable with that of subcomponent C1q (96.5%).

In addition, subcomponent C1q contains disulphide bonds in its polypeptide structure (Yonemasu & Stroud, 1972; Reid, 1976; Reid & Porter, 1976). The overall chemical characteristics of the subcomponent C1q molecule, obtained from results presented here, and including the presence of disulphide bonds, are similar to those of the collagenous constituents in human glomerulus, aortic intima and in bovine anterior lens capsule with the higher molecular weight.

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