Studies on the Structure and Activity of Rabbit C1q (a Subcomponent of the First Component of Complement)

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1. The subunit structure of rabbit subcomponent C1q was examined in a previous publication (Reid *et al.*, 1972). The present paper describes some aspects of the structure of the polypeptide chains derived from the molecule. 2. The three polypeptide chains, produced by performic oxidation, of rabbit subcomponent C1q were isolated by ion-exchange chromatography in 8M-urea on DEAE-cellulose. 3. Each chain was found to contain 15–18% glycine and significant amounts of the amino acids hydroxyproline and hydroxylysine. 4. By means of collagenase digestion it was shown that all three chains of rabbit subcomponent C1q contain collagen-like sequences of amino acids which constitute about 40% of each chain. 5. By use of carboxypeptidase A it was established, indirectly, that the collagen-like sequences, in one of the chains, are probably located near, or at, the *N*-terminal end of the chain. 6. Collagenase digestion and heating at 52°C (but not at 49°C) caused rapid loss of native rabbit subcomponent C1q haemolytic activity.

Subcomponent C1q has been shown to contain binding sites for certain classes of immunoglobulin (Müller-Eberhard & Kunkel, 1961; Augener *et al.*, 1971). On interaction with aggregated immunoglobulin, subcomponent C1q brings about the activation of the first component of complement, which has been shown to be a calcium-dependent complex of the three subcomponents C1q, C1r and C1s in humans (Lepow *et al.*, 1963), guinea pigs (Sassano *et al.*, 1972) and rabbits (Lowe, 1973). Human subcomponent C1q (Yonemasu *et al.*, 1971; Calcott & Müller-Eberhard, 1972; Reid *et al.*, 1972) and rabbit subcomponent C1q (Reid *et al.*, 1972; Volankis & Stroud, 1972) have both been well characterized. Each has been shown to have an unusual amino acid composition consistent with there being a region of collagen-like amino acid sequence in the molecule. This has now been confirmed by sequence studies on the three polypeptide chains of human subcomponent C1q (Reid, 1974, and unpublished work). The present paper shows that each of the three chains of rabbit subcomponent C1q has a collagen-like sequence of amino acids constituting about 40% of the chain, and which is probably located in the *N*-terminal portion of the chain. In addition, it has been shown that the haemolytic activity of native rabbit subcomponent C1q is rapidly destroyed by collagenase digestion and heating at 52°C but not by heating at 49°C.

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Materials

Phenyl isothiocyanate, *N*-ethylmorpholine, trifluoroacetic acid, *N*-ethylmaleimide, amino acids and dansyl chloride were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. The chemicals used in the Edman degradation procedures were purified and redistilled before use.

Silica-gel Chromogram sheets (type 6061) were supplied by Eastman Kodak, Kirkby, Liverpool, U.K.

Sephadex G-200 and G-25 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Carboxypeptidase A, trypsin and collagenase (CLSPA, chromatographically purified) were obtained from Worthington Biochemical Corp., Freehold, N.J., U.S.A.

Methods

Isolation of rabbit subcomponent C1q

Rabbit subcomponent C1q was isolated as previously described (Reid *et al.*, 1972), except that 55 mM-sodium phosphate–1 mM-EDTA buffer, pH 7.4, was used in the DEAE-cellulose step and 230 mM-sodium acetate buffer, pH 5.2, was used as the equilibrating and starting buffer in the CM-cellulose chromatography.

Collagenase digestion of native rabbit subcomponent C1q

Chromatographically purified Worthington collagenase was further purified by gel filtration on Sepha-

dex G-200 as described by Peterkofsky & Diegelmann (1971). Purified collagenase, utilized under the conditions described below, is specific for Y-Gly bonds in the repeating sequence (Gly-X-Y)_n, as shown by Reid (1974) in sequence studies on collagen-like peptides isolated from human subcomponent C1q. The purified collagenase (500 µg) was added to native rabbit subcomponent C1q (10mg) that had been dissolved in 5.0ml of 100mM-Tris-acetate buffer, pH 8.4, made 5mM with respect to calcium acetate. The digest was incubated at 37°C, and samples (50 µl) were taken, at time-intervals from 0.25 to 24h, and were diluted in ice-cold 50mM-Tris-40mM-HCl-0.15mM-CaCl₂-25mM-NaCl-146mM-sucrose buffer, pH 7.4 (0.45ml) and stored at -70°C until assayed for subcomponent C1q haemolytic activity as described by Reid *et al.* (1972). After 24h at 37°C, the digest was spun at 30000g for 30min at 4°C, and the supernatant and precipitate were examined by polyacrylamide-gel electrophoresis in buffers containing 0.1% (w/v) sodium dodecyl sulphate and by amino acid analysis. The supernatant was fractionated by gel filtration on Sephadex G-25 in 100mM-acetic acid and the fractions were subjected to amino acid analysis.

Effect of heat treatment on rabbit subcomponent C1q haemolytic activity

The sensitivity of rabbit subcomponent C1q haemolytic activity after incubation for 60min at temperatures ranging from 44° to 56°C was investigated. The treated samples were compared with a sample kept at 4°C for 60min.

Isolation of the oxidized A, B and C chains of rabbit subcomponent C1q

The performic oxidation of rabbit subcomponent C1q and the isolation of the oxidized A, B and C chains on DEAE-cellulose, in 8M-urea, were performed as previously described for human subcomponent C1q (Reid *et al.*, 1972).

Enzymic digestion of the oxidized chains and fragments derived from the oxidized chains

Trypsin. Trypsin was treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (L-1-chloro-4-phenyl-3-tosylamidobutan-2-one) to remove chymotryptic activity as described by Kostka & Carpenter (1964). The treated trypsin (100 µg in 100 µl of 1mM-HCl) was added to the sample of oxidized subcomponent C1q or the isolated A, B or C chain (2mg), which was suspended in 0.5ml of 200mM-NH₄HCO₃ buffer, pH 8.1. Digestion was carried out at 37°C for 5h. The digest was freeze-dried, dissolved in 200mM-NH₄HCO₃ buffer, pH 8.1 (200 µl), and

examined by electrophoresis at pH 6.5, in one dimension, followed by chromatography in the second dimension on silica-gel chromatogram sheets essentially as described by Sargent & Vadlamudi (1968).

Collagenase. Purified collagenase (400 µg) in 700 µl of 100mM-Tris-acetate buffer, pH 8.4, made 5mM with respect to calcium acetate and 0.25mM with respect to N-ethylmaleimide, was added to the oxidized A, B or C chain (4.0mg), and the digestion was performed at 37°C for 24h. The digest was spun at 30000g for 30min at 4°C. The precipitate was washed with digestion buffer (0.5ml) and spun down, as before, and the two supernatants were pooled. The precipitate and supernatant were examined by polyacrylamide-gel electrophoresis in buffers containing 0.1% (w/v) sodium dodecyl sulphate and by amino acid analysis. The supernatant of each digest was fractionated by gel filtration on Sephadex G-25 in 50mM-NH₃, and the fractions obtained were examined by amino acid analysis. Two of these fractions were also subjected to four steps of the quantitative Edman degradation procedure, carried out essentially as described by Doppeide *et al.* (1967). The precipitate, and one of the supernatant fractions from the collagenase digest of each of the oxidized chains, were digested with carboxypeptidase A.

Carboxypeptidase A. Peptide (0.02–0.05 µmol) was dissolved in 100mM-sodium phosphate buffer, pH 7.4 (90 µl), made 0.1% (w/v) with respect to sodium dodecyl sulphate. Enzyme (50 µg in 10 µl of digestion buffer) was added and the mixture was incubated at 37°C for 17h. The reaction was stopped by acidification with 0.1M-HCl and the digest was examined by amino acid analysis. A control sample of carboxypeptidase A incubated alone was included.

Haemolytic assays, quantitative amino acid analysis, polyacrylamide-gel electrophoresis in sodium dodecyl sulphate, N-terminal analysis, electrophoresis and chromatography

All these methods were performed as described by Reid *et al.* (1972).

Results

Collagenase digestion of native rabbit subcomponent C1q

Digestion of rabbit subcomponent C1q with collagenase at 37°C caused a rapid decrease in haemolytic activity as compared with the control sample held at 37°C (Table 1). Incubation of bovine serum albumin with collagenase, under the same conditions, gave no degradation products, confirming that the collagenase was not contaminated by other proteolytic enzymes.

Table 1. *Collagenase digestion of rabbit subcomponent C1q*

Subcomponent C1q (750 H₅₀ units/ml) was digested with collagenase and assayed for residual haemolytic activity as described in the text. A control sample of subcomponent C1q, incubated without collagenase, was subjected to the same digestion and assay procedures.

Incubation time (h)	Haemolytic activity remaining (%)	
	Digest	Control
0	100	100
0.5	90	98
1	11	97
2	5	97
4	3	97
8	3	97
24	3	85

Table 2. *Amino acid compositions of rabbit subcomponent C1q and the precipitate and supernatant from the collagenase digest of rabbit subcomponent C1q*

Full experimental details are given in the text. The results are expressed as amino acid residues/100 residues. Samples were hydrolysed at 110°C for 48 h, and no corrections for destruction of threonine and serine or slow release of valine and isoleucine have been made. N.D., Not determined.

Amino acid	Amino acid composition (residues/100 residues)		
	Rabbit subcomponent C1q	Collagenase digest	
		Precipitate	Supernatant
Hyp	4.9	Nil	8.0
Asp	8.3	10.1	6.6
Thr	4.1	6.9	3.8
Ser	4.7	5.1	3.5
Glu	8.5	8.3	7.8
Pro	6.4	4.7	8.7
Gly	17.6	9.8	22.9
Ala	5.6	4.8	6.9
Val	5.9	12.5	6.0
Cys	2.5	N.D.	N.D.
Met	1.0	1.1	0.6
Ile	3.4	2.5	4.1
Leu	5.8	7.9	5.1
Tyr	2.8	5.6	0.8
Phe	4.8	7.2	1.5
His	2.5	4.2	2.1
Hyl	1.4	0.5	3.1
Lys	3.6	4.2	3.8
Arg	5.5	4.7	5.6
Trp	0.5	N.D.	N.D.

The loss of haemolytic activity coincided with the appearance of a precipitate that accounted for 30–40% of the starting material. Amino acid analysis of the supernatant and precipitate (Table 2) showed that the supernatant had been enriched in hydroxy-

Table 3. *Sensitivity of rabbit subcomponent C1q haemolytic activity to temperature*

Subcomponent C1q (3100 H₅₀ units/ml) was incubated at temperatures from 44° to 56°C for 60 min and the residual haemolytic activity compared with that of a control sample kept at 4°C.

Temperature of incubation (°C)	Residual haemolytic activity (%)
44.0	100
45.8	100
48.5	100
50.0	45
52.0	0
54.0	0
56.0	0

lysine, hydroxyproline and glycine when compared with the amino acid analysis of the intact rabbit subcomponent C1q (Table 2). On the other hand, the precipitate contained no hydroxyproline and little hydroxyllysine, and had a relatively low glycine content.

Since the collagenase digestion separated the rabbit subcomponent C1q into collagen-like and non-collagen-like fractions, it was decided to assay the haemolytic activity of intact rabbit subcomponent C1q (0.6 nmol) in the presence of either the supernatant material (0.49 nmol) or the resuspended precipitate (0.26 nmol). In each case there was no detectable inhibition of subcomponent C1q haemolytic activity under the conditions used.

Effect of heat treatment on rabbit subcomponent C1q

Over 90% of rabbit subcomponent C1q was precipitated on heat treatment at 56°C for 30 min, causing complete loss of haemolytic activity. The subcomponent C1q haemolytic activity showed a remarkably sharp decrease over the temperature range 49–52°C (Table 3). It was notable that no subcomponent C1q haemolytic activity was lost after incubation at temperatures between 25° and 48°C for 60 min before assay.

Subunit and chain structure of rabbit subcomponent C1q

The determination of the molecular weights of intact rabbit subcomponent C1q and its non-covalently linked subunits has been described previously (Reid *et al.*, 1972). These results are considered in the Discussion section in the light of the new results given in the present paper on the structure of the polypeptide chains of rabbit subcomponent C1q.

Fig. 1. Separation of the oxidized chains of rabbit subcomponent C1q

Oxidized subcomponent C1q (15mg) was applied to a column (1 cm $\times$ 35 cm) of DEAE-cellulose and eluted with 3 mM-sodium phosphate-8 M-urea buffer, pH 7.8, up to tube 30, and with 67 mM-sodium phosphate-8 M-urea buffer, pH 7.8, after tube 31.

The separation of the oxidized chains of rabbit subcomponent C1q is shown in Fig. 1. The recovery of protein from the column, as estimated by the absorbance at 280 nm in five different experiments, was always about 70% of that applied. The average percentage of each chain, by weight, over five experiments was: A chain, 28%; B chain, 37%; C chain, 35%. The separated oxidized A, B and C chains of rabbit subcomponent C1q run on polyacrylamide gels in buffers containing sodium dodecyl sulphate are shown in Fig. 2. The amino acid composition of each chain is shown in Table 4. All three chains contain hydroxyproline and hydroxylysine residues and a high glycine value (Table 4) similar to that found in the intact rabbit subcomponent C1q (Table 2). Indications that the three chains may differ in amino acid sequence can be seen from comparison of the arginine content of each chain. This was markedly higher in the A chain in comparison with the B and C chains. On the other hand, the histidine and tyrosine contents of the C chain were significantly higher than those of the B and A chains. The threonine/serine and isoleucine/leucine ratios were also different in each of the chains (Table 4). The amino acid compositions of the three chains expressed as mol. of residues/mol. of each chain (Table 4) were calculated from the number of histidine residues found per chain (see below) and assuming a molecular weight of approx. 23 000 per chain (Reid *et al.*, 1972). The number of tryptic peptides seen was in good agreement with the number predicted on a basis of the arginine, lysine and hydroxylysine contents, except for the A chain, where the number of tryptic peptides seen was lower than expected. Sequence studies

Fig. 2. Polyacrylamide-gel electrophoresis on 10% gels, in the presence of 0.1% sodium dodecyl sulphate, of the oxidized chains and the precipitates from the collagenase digest of each chain

Experimental details are given in the text. Bovine serum albumin was included with each sample to allow accurate comparison of the gels. (a) A chain; (b) B chain; (c) C chain; (d) A chain collagenase-digest precipitate; (e) B chain collagenase-digest precipitate; (f) C chain collagenase-digest precipitate.

on the A chain of human subcomponent C1q (Reid, 1974) have shown that the peptide bonds C-terminal to hydroxylysine residues in this chain are resistant to trypsin and that this is probably due to the presence of carbohydrate on these residues.

N-Terminal analysis of the separated oxidized chains of rabbit subcomponent C1q

With the use of the dansyl technique serine was detected as the only *N*-terminal amino acid of the oxidized C chain. No detectable *N*-terminal amino acids were found for either the A or B chains.

C-Terminal analysis of the separated oxidized chains of rabbit subcomponent C1q

Treatment of the oxidized A chain of rabbit subcomponent C1q yielded the C-terminal amino acids shown in Table 5. Alanine was the major C-terminal amino acid of each of the three chains and of the oxidized subcomponent C1q digested under identical conditions. From the yields of amino acids obtained from each chain, the tentative C-terminal sequence of the A chain might be: -Ile-Phe-Ala. The yields of amino acids from the B and C chains

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

The results are expressed as residues/100 residues and as mol of residues/mol assuming the integral number of histidine residues shown. Full experimental details are given in the text. Samples were hydrolysed at 110°C for 48h and no corrections for destruction of threonine and serine or slow release of valine and isoleucine have been made. N.D., Not determined. Abbreviations used here and in Table 6: Cys (O₃H), Cysteic acid; Met(O₂), methionine sulphone.

Amino acid	Amino acid composition					
	(residues/100 residues)			(mol of residues/mol) assuming:		
	A	B	C	His = 3 A	His = 5 B	His = 7 C
Cys(O ₃ H)	2.2	2.5	2.9	4.1	5.0	5.6
Hyp	9.4	6.3	2.8	17.7	12.5	5.3
Asp	6.7	8.3	10.2	12.6	16.4	19.8
Met(O ₂)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr	3.2	5.1	7.1	6.0	10.2	13.9
Ser	3.6	4.8	4.7	6.8	9.6	9.2
Glu	7.8	9.9	8.7	14.6	19.7	16.9
Pro	7.0	6.8	6.1	13.1	13.4	11.9
Gly	18.0	18.3	15.0	33.8	36.5	29.2
Ala	5.9	6.5	4.9	11.1	12.9	9.5
Val	5.1	4.9	7.5	9.5	9.9	14.5
Ile	4.1	4.1	2.1	7.7	8.0	5.3
Leu	3.8	6.1	7.3	7.1	12.2	14.2
Tyr	1.7	2.4	3.3	3.2	4.7	6.4
Phe	5.0	3.9	4.7	9.4	7.6	9.1
His	1.6	2.5	3.6	3.0	5.0	7.0
Hyl	2.0	2.3	0.8	3.7	4.6	1.6
Lys	3.8	3.7	3.7	7.2	7.4	7.3
Arg	8.9	3.8	4.2	16.7	7.6	8.2
No. of amino acids (taken to nearest integer)				198	201	195
Estimated mol. wt.				21800	22100	21500
No. of trypsin-susceptible bonds expected				27	20	17
No. of trypsin peptides seen				18	18	15

Table 5. *C-Terminal analysis of the oxidized A chain and precipitate isolated from a collagenase digest of the oxidized A chain*

The results are given as nmol of amino acid released from the intact chain (43nmol) or the collagenase precipitate (40nmol) after correction for the carboxypeptidase A control. The values of all amino acids omitted from the Table were nil. Full experimental details are given in the text.

Amino acid	Amino acid released (nmol)	
	Intact A chain	Collagenase precipitate
Asp	1.3	4.4
Thr	Nil	4.5
'Ser'*	4.4	6.5
Glu	1.1	4.3
Gly	4.3	6.1
Ala	30.6	26.8
Val	3.2	7.0
Ile	14.1	9.4
Leu	6.0	8.6
Tyr	0.9	1.7
Phe	19.1	18.4

* Glutamine and asparagine are eluted in the same position as serine under the conditions used for amino acid analysis.

were very low, and therefore they are not included in Table 5. This might be due to the relative insolubility of these chains in the 100mM-sodium phosphate-0.1% sodium dodecyl sulphate buffer, pH 7.2, used for the digestion.

Tryptic digestion of rabbit subcomponent C1q and the separated oxidized chains

Peptide 'mapping' of trypsin-digested oxidized rabbit subcomponent C1q on thin-layer silica-gel plates indicated that there were 32 or 33 ninhydrin-positive peptides, of which 11 or 12 were positive when stained for histidine. Comparison of the peptide 'map' of trypsin-digested oxidized rabbit subcomponent C1q with those obtained in a similar manner for separated oxidized chains showed that of the 33 peptides found in the whole molecule 18 were found in the A chain, 17 in the B chain and 15 in the C chain. The tryptic peptide 'maps' of the oxidized A, B and C chains shared some common peptides, mostly in the neutral region. When considered overall, however, the pattern of the 'map' obtained for each chain was different from the others. This is especially apparent for the peptides staining for histidine. The A chain

had three peptides that stained strongly for histidine and one that stained faintly; by comparison, the B chain had five to six peptides staining strongly for histidine and the C chain had seven peptides staining strongly for histidine.

Collagenase digestion of the separated oxidized chains of rabbit subcomponent C1q

Digestion of the separated oxidized chains of rabbit subcomponent C1q with collagenase resulted in the appearance of a precipitate that accounted for about 30% of the recovered material for both the A and B chains and for 50% of that recovered for the C chain. Examination of the precipitate and supernatant of each digest by polyacrylamide-gel electrophoresis in the presence of 0.1% sodium dodecyl sulphate showed that the precipitated material from the A chain was made up of one major and two minor fragments, in addition to some undigested A-chain material; the apparent molecular weight of the major one was 15000 and those of the minor ones were 13000 and 12000. The precipitate of the B chain gave one major fragment, of apparent molecular weight 12000, along with a small amount of some high-molecular-weight-component bands, and that of the C chain gave a mixture of fragments of apparent molecular weights between 12000 and 8500 (Fig. 2).

The supernatant fractions appeared to be made up primarily of lower-molecular-weight material that could not be seen on polyacrylamide gels (10%) run in 0.1% sodium dodecyl sulphate. Fractionation of the supernatant material on Sephadex G-25 resulted in the separation of four fractions accounting for approx. 10, 20, 29 and 4% respectively of the starting material for each chain. Amino acid analysis of these fractions indicated that the first fraction from each chain was non-collagenous, whereas the other three fractions all had high hydroxyproline, hydroxylysine and glycine contents and appeared to be made up of collagen-like material. The amino acid analyses of the precipitate and unfractionated supernatant obtained from the collagenase digests of the A, B and C chains are shown in Table 6. In all three chains the precipitate had relatively low glycine and hydroxylysine contents and contained no hydroxyproline. In contrast, the supernatant was enriched in these amino acids as compared with the undigested chain (Tables 4 and 6).

C-Terminal analysis of the precipitate obtained by collagenase digestion of the oxidized A chain of rabbit subcomponent C1q

The major C-terminal residues found in the precipitate of the collagenase digest of the oxidized A chain

Table 6. *Amino acid compositions of the precipitates and unfractionated supernatant of the collagenase digests of the oxidized A, B and C chains of rabbit subcomponent C1q*

The results are expressed as residues/100 residues. Full experimental details are given in the text. Samples were hydrolysed at 110°C for 48h and no corrections for destruction of threonine and serine or slow release of valine and isoleucine have been made. N.D., Not determined.

Amino acid	Amino acid composition (residues/100 residues)					
	Precipitate			Supernatant		
	A	B	C	A	B	C
Cys(O ₃ H)	1.9	1.7	2.4	0.9	0.9	1.3
Hyp	Nil	Nil	Nil	9.0	10.9	7.7
Asp	10.1	9.6	10.4	7.1	7.2	8.4
Met (O ₂)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Thr	5.4	5.0	7.7	3.2	4.0	6.1
Ser	5.8	4.9	4.7	3.9	3.8	4.5
Glu	10.0	8.9	8.6	6.2	7.0	8.2
Pro	3.4	3.0	1.7	4.2	3.5	3.3
Gly	13.8	10.6	11.8	25.7	25.1	19.6
Ala	5.9	5.1	5.5	6.7	6.9	4.9
Val	10.6	6.6	10.9	3.8	2.8	5.3
Ile	5.0	3.4	2.5	3.7	5.0	3.8
Leu	5.3	13.7	9.4	3.8	5.0	5.3
Tyr	3.5	5.7	5.5	1.6	2.0	2.8
Phe	7.7	6.0	6.9	2.8	2.6	3.3
His	2.5	3.4	3.9	2.2	3.4	4.2
Hyl	0.5	0.6	0.2	3.4	4.6	2.0
Lys	3.3	6.7	4.0	4.8	3.7	4.2
Arg	5.4	4.8	4.0	7.2	2.1	5.2

Table 7. *Sequential Edman degradation of the supernatant fractions obtained by collagenase digestion of the oxidized A, B and C chains*

Full experimental details are given in the text.

Fraction	Glycine content (residues/100 residues)	Change in glycine content (%)			
		Step 0–Step 1	Step 1–Step 2	Step 2–Step 3	Step 3–Step 4
A III	26.4	–25.5	+ 9.1	+29.0	–45.0
B III	29.8	–34.5	+25.0	+30.1	–52.4
C III	23.2	–15.0	+ 2.6	+24.1	–17.6

were identical with those found for the undigested chain (Table 5). This suggests that the precipitate from the collagenase digest is derived from a non-collagen-like C-terminal portion of the A chain.

Sequential Edman degradation of the collagen-like supernatant fractions produced by collagenase digestion of the oxidized A, B and C chains

Three of the collagen-like fractions obtained by gel filtration of the collagenase-digest supernatants, one from each chain, were subjected to four steps of the sequential Edman degradation procedure to see whether evidence for the presence of repeating sequences of the type (Gly-X-Y)_n could be established. Only the glycine values are considered in Table 7, since if a sequence of the type (Gly-X-Y)_n were present only the glycine values would behave in a predictable manner. The specificity of collagenase is such that only N-terminal glycine is found in a mixture of peptides derived from the digestion of a collagen-like sequence. The significant loss of glycine in step 1 of the Edman degradation, followed by a rise in glycine content at steps 2 and 3 and finally followed by a marked fall in glycine content at step 4 (Table 3), is what would be theoretically expected for a mixture of peptides composed mainly of the repeating triplet (Gly-X-Y)_n. The value *n* would lie between 2 and 6 as judged by the elution volumes on Sephadex G-25. This information therefore suggests that sequences of the type (Gly-X-Y)_n are present in each of the chains of rabbit subcomponent C1q and is consistent with the ability of collagenase to digest these chains.

Discussion

The importance of the collagen-like regions in rabbit subcomponent C1q is shown by the susceptibility of subcomponent C1q haemolytic activity to digestion with purified collagenase (Table 1). The inability of both the precipitate and supernatant fraction from the collagenase digest to inhibit the haemolytic activity of intact subcomponent C1q is perhaps not surprising, since the precipitate behaved as a suspension on dilution in the assay buffers and the supernatant was composed of mainly small

peptides that were retained on Sephadex G-25. The sharp loss of haemolytic activity between 48° and 52°C may also reflect the importance of the collagenous region in rabbit subcomponent C1q. Comparison of the temperature-dependence of subcomponent C1q haemolytic activity with the 'melting' temperature, at neutral pH, of native *Ascaris* collagen, which is one of the few collagens reported to contain disulphide bridges (Haushka & Harrington, 1970), indicates that they have the same sensitivity to small temperature changes in the region of 48–52°C.

The three polypeptide chains of rabbit subcomponent C1q isolated after performic acid oxidation were obtained in equimolar yield. Amino acid analyses of the three chains (Table 4) suggest that each chain may have a region of collagen-like sequence. The high arginine content of the A chain and the high tyrosine and histidine contents of the C chain, together with the different threonine/serine and isoleucine/leucine ratios in each chain, indicates that the three chains are not identical. Moreover, when the results are expressed as mol of residues/mol (Table 4), the three chains differ in length by, at most, six or seven amino acid residues. These results suggest that none of the chains isolated is derived from another as a result of degradation. N-Terminal and C-terminal analysis of the chains also showed differences between the three chains, as did tryptic peptide 'mapping'.

Digestion of the three chains with purified collagenase resulted in the appearance of a precipitate containing the non-collagenous portion of each chain and a supernatant fraction that appeared to contain collagen-like peptides as determined by amino acid analysis (Table 6). C-Terminal analysis of the precipitate obtained by collagenase digestion of the A chain indicates that the major C-terminal residues are identical with those found for the intact chain (Table 5). This suggests that the collagenase precipitates are derived from a non-collagenous C-terminal portion of each chain. Molecular-weight determinations, by the use of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate, indicate that this region probably extends for approximately one-half the length of the chain. Sequential Edman degradation of fractions obtained by fractionation of the supernatant material from the

collagenase digestion of each of the three chains provided tentative evidence that there are repeating sequences of the type (Gly-X-Y) in each chain. The evidence given here suggests that the collagen-like sequences will be found at, or near, the *N*-terminal end of each chain, as has been shown to be the case in human subcomponent C1q (Reid, 1974).

The results presented here and previously (Reid *et al.*, 1972) suggest a close structural similarity between human and rabbit subcomponents C1q, as indicated by the similarity between their physical and chemical properties and complete interchangeability in the haemolytic assay system. The results obtained by Reid *et al.* (1972) and Calcott & Müller-Eberhard (1972) suggest that human subcomponent C1q of molecular weight 410000 is composed of probably six non-covalently linked subunits, each of which is made up of three non-identical polypeptide chains each of approximate molecular weight 23000. One anomaly remains to be explained, namely the apparent difference between human and rabbit subcomponents C1q when examined, before reduction and alkylation, on polyacrylamide gels in the presence of sodium dodecyl sulphate. Whereas human subcomponent C1q gave only one major band staining for protein, rabbit subcomponent C1q consistently gave two major components (Reid *et al.*, 1972), and similar results have been reported for rabbit subcomponent C1q isolated by a different method (Volankis & Stroud, 1972).

The presence of two types of non-covalently linked subunit in rabbit subcomponent C1q may be due to the loss of carbohydrate from only a certain percentage of the non-covalently linked subunits, as pointed out previously (Reid *et al.*, 1972). It is well known that the carbohydrate content of a protein affects its mobility on electrophoresis in polyacrylamide in the presence of sodium dodecyl sulphate (Shubert, 1970; O'Daly & Cebra, 1971). Amino acid analysis of the two bands obtained by electrophoresis of rabbit subcomponent C1q indicates that both components have an amino acid composition very similar to that of the intact subcomponent C1q (Lowe, 1973). However, owing to the technical difficulties involved in eluting the protein bands from polyacrylamide

gels, the results are not sufficiently quantitative to indicate whether or not the two components have identical amino acid compositions. The greater lability of rabbit subcomponent C1q haemolytic activity during handling (and its tendency to form inactive aggregates) suggests that rabbit C1q may be susceptible to degradation during the purification procedure, and this could explain the appearance of variable amounts of the lower-molecular-weight component.

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