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Subunit Composition and Structure of Subcomponent C1q of the First Component of Human Complement

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1. Unreduced human subcomponent C1q was shown by electrophoresis on polyacrylamide gels run in the presence of sodium dodecyl sulphate to be composed of two types of non-covalently linked subunits of apparent mol.wts. 69000 and 54000. The ratio of the two subunits was markedly affected by the ionic strength of the applied sample. At a low ionic strength of applied sample, which gave the optimum value for the 54000-apparent-mol.wt. subunit, a ratio of 1.99:1.00 was obtained for the ratio of the 69000-apparent-mol.wt. subunit to the 54000-apparent-mol.wt. subunit. The amount of the 54000-apparent-mol.wt. subunit detected in the expected position on the gel was found to be inversely proportional to increases in the ionic strength of the applied sample. 2. Human subcomponent C1q on reduction and alkylation, or oxidation, yields equimolar amounts of three chains designated A, B and C [Reid *et al.* (1972) *Biochem. J.* **130**, 749–763]. The results obtained by Yonemasu & Stroud [(1972) *Immunochemistry* **9**, 545–554], which showed that the 69000-apparent-mol.wt. subunit was a disulphide-linked dimer of the A and B chains and that the 54000-apparent-mol.wt. subunit was a disulphide-linked dimer of the C chain, were confirmed. 3. Gel filtration on Sephadex G-200 in 6.0M-guanidinium chloride showed that both types of unreduced subunit were eluted together as a single symmetrical peak of apparent mol.wt. 49000–50000 when globular proteins were used as markers. The molecular weights of the oxidized or reduced A, B and C chains have been shown previously to be very similar, all being in the range 23000–24000 [Reid *et al.* (1972) *Biochem. J.* **130**, 749–763; Reid (1974) *Biochem. J.* **141**, 189–203]. 4. It is proposed that subcomponent C1q (mol.wt. 410000) is composed of nine non-covalently linked subunits, i.e. six A–B dimers and three C–C dimers. 5. A structure for subcomponent C1q is proposed and is based on the assumption that the collagen-like regions of 78 residues in each of the A, B and C chains are combined to form a triple-helical structure of the same type as is found in collagens.

The chemical evidence given in the preceding paper (Reid, 1976) shows that the complement subcomponent C1q contains disulphide-bonded dimers of A and B chains and of C chains. Each chain contains about 190 amino acid residues, and there is 12, 8 and 4% carbohydrate in the A, B and C chains respectively (Reid *et al.*, 1972; Reid, 1974). Thus the dimers would be expected to have mol.wts. of approx. 49000 for the A–B dimer and approx. 46000 for the C–C dimer.

Yonemasu & Stroud (1972), from the behaviour of human subcomponent C1q in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, suggested that two subunits were present of apparent mol.wts. 60000 and 42000, whereas Reid *et al.* (1972) found only one component, with an apparent mol.wt. of 69000. Neither estimates agree with the chemical data, and, on re-examination by polyacrylamide-gel electrophoresis run in the presence of sodium dodecyl sulphate, it has become clear that two subunits are present, as reported by Yonemasu & Stroud (1972), but with apparent mol.wts., with unreduced marker proteins, of 69000 and 54000, which are close to the values reported by Knobel *et al.* (1975).

Hence the anomalous behaviour of these subunits has been re-investigated by using electrophoresis in polyacrylamide gels run in buffers containing sodium dodecyl sulphate. Another estimate of size has been made from their rate of elution from Sephadex G-200 in 6M-guanidinium chloride. The latter method gives values much closer to that expected from the chemical data.

On the assumption that the chains will associate through their collagenous sections, a structure is proposed that agrees with the electron-microscope pictures of subcomponent C1q and with the chemical and physical data.

Materials and Methods

Isolation of human subcomponent C1q

Human subcomponent C1q was isolated as described by Reid (1974).

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was performed essentially as described by Fairbanks *et al.* (1971). Samples were run on 5.6 or 7% polyacrylamide gels in 40mM-Tris/20mM-sodium acetate/2mM-EDTA/0.2% (w/v) sodium dodecyl sulphate buffer, pH7.4. The gels were always pre-run for at least 30min. Samples were prepared by incubation at 37°C for 2h, or at 100°C for 2–4min, in buffers of various ionic strengths, which were always 4M with respect to urea and 1% (w/v) with respect to sodium dodecyl sulphate unless stated otherwise. The gels were stained with 2.5% (w/v) Coomassie Brilliant Blue in methanol/acetic acid/water (91:18:91, by vol.) and de-stained electrophoretically in 7% (v/v) acetic acid.

Gel filtration on Sephadex G-200 in 6M-guanidinium chloride

Samples (1.0ml) of the standard marker proteins transferrin (mol.wt. 82000), bovine serum albumin (mol.wt. 65000), immunoglobulin G heavy chain (mol.wt. 50000), immunoglobulin G light chain (mol.wt. 23000) and lysozyme (mol.wt. 13000) were run on a Sephadex G-200 column (1.8cm×78cm) equilibrated with 6M-guanidinium chloride, pH7.0, and run, by upward flow, at a rate of 5ml/h at room temperature (22°C). Fractions of volume 3ml were collected by a siphon. Samples of subcomponent C1q were mixed with an equal volume of 6M-guanidinium chloride, pH7.0, containing iodoacetamide (10mg/ml), and then dialysed for 20h against the buffer used to equilibrate the column before application to the column.

Results

Polyacrylamide-gel electrophoresis of human subcomponent C1q: a re-examination of the subunit structure of subcomponent C1q

In previous experiments performed in this laboratory (Reid *et al.*, 1972) unreduced subcomponent C1q ran as a single major band of apparent mol.wt. 69000 on polyacrylamide gels in buffers containing sodium dodecyl sulphate and with unreduced globular marker proteins as standards (Fig. 1*a*). In the present study it was observed that the behaviour of subcomponent C1q was influenced markedly by the ionic strength of the solution in which the sample is loaded on to the gel. A second major band of apparent mol.wt. 54000 becomes apparent if the subcomponent C1q is loaded

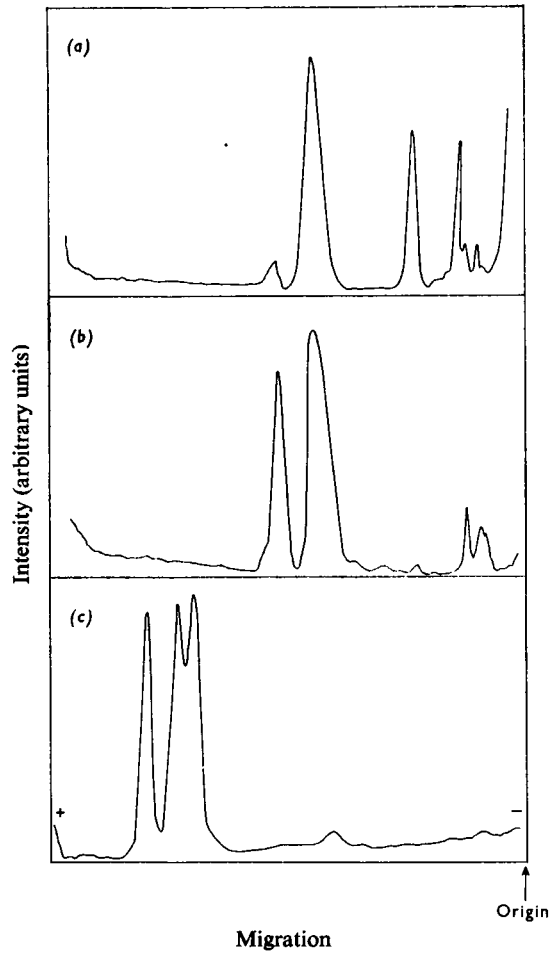
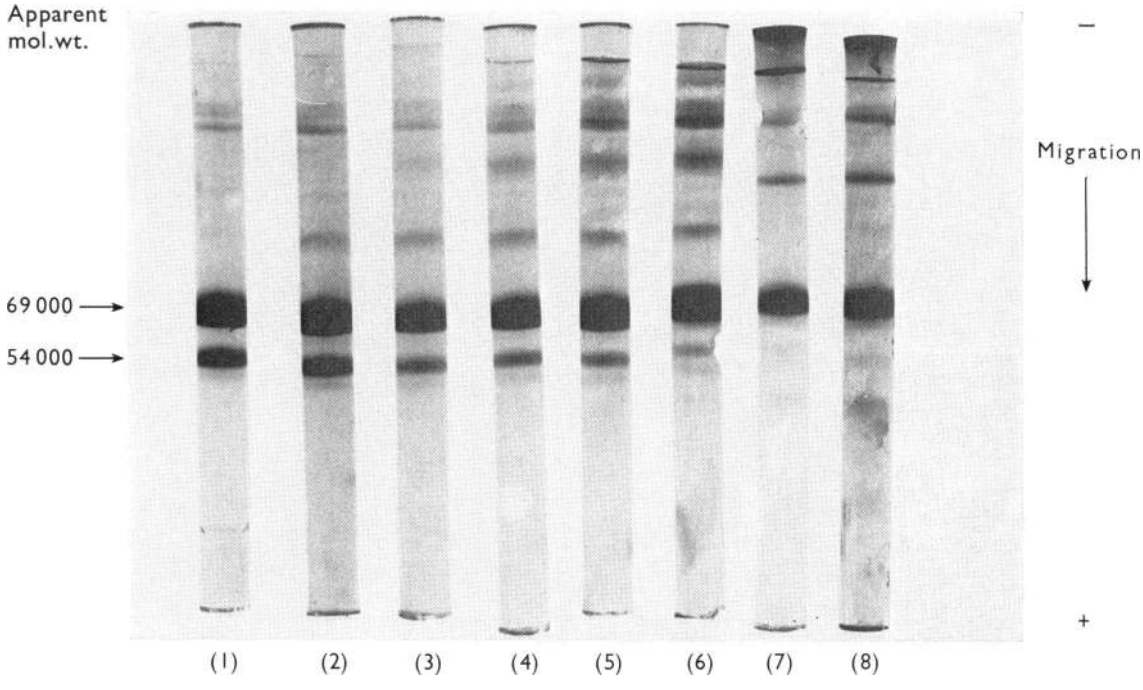


Fig. 1. *Scans of polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate of subcomponent C1q*

The samples were run on 7% acrylamide gels in buffer containing sodium dodecyl sulphate. (*a*) Unreduced subcomponent C1q; the ionic strength of the applied sample was 0.33. (*b*) Unreduced subcomponent C1q; the ionic strength of the applied sample was 0.05. (*c*) Reduced and alkylated subcomponent C1q; the ionic strength of the applied sample was 0.07.

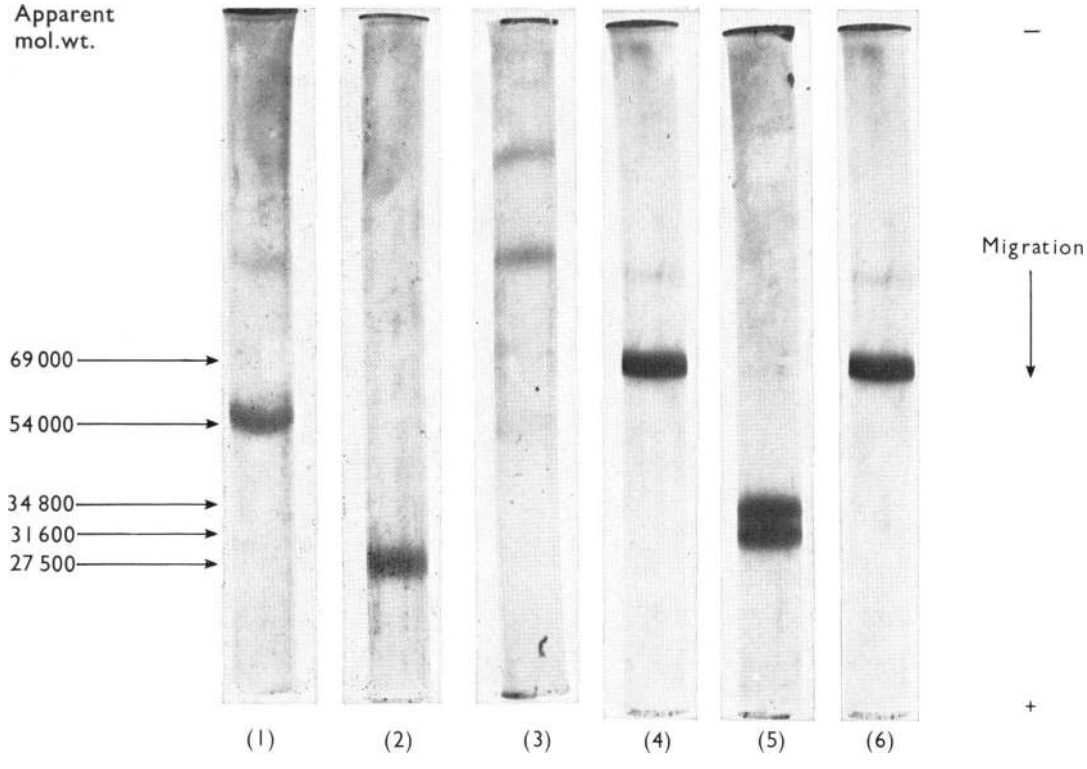
in solution of low ionic strength (Fig. 1*b*), and in a sample of ionic strength 0.05 the ratio of the 69000-apparent-mol.wt. subunit to the 54000-apparent-mol.wt. subunit was found to be 1.99:1.00 (Fig. 1*b*). The ratio was determined as described in the preceding paper (Reid, 1976). Plate 1 shows that the amount of the 54000-apparent-mol.wt. subunit found was inversely proportional to the ionic strength of the solution in which the samples were loaded. Prior heat-



EXPLANATION OF PLATE 1

Electrophoresis on 5.6% *polyacrylamide gels, run in buffer containing* 0.2% *sodium dodecyl sulphate, of subcomponent C1q with increasing ionic strengths of the applied sample*

(1) Unreduced human subcomponent C1q; sample in phosphate buffer, $I = 0.05$ mol/l, pH 7.4, containing 4 M-urea, 1% (w/v) sodium dodecyl sulphate and 20 mM-iodoacetamide. (2) Sample identical with (1) except that it was 50 mM with respect to NaCl and 20 mM with respect to Tris/HCl buffer, pH 7.4. (3) Sample identical with (1) except that it was 100 mM with respect to NaCl and 40 mM with respect to Tris/HCl buffer, pH 7.4. (4) Sample identical with (1) except that it was 150 mM with respect to NaCl and 60 mM with respect to Tris/HCl buffer, pH 7.4. (5) Sample identical with (1) except that it was 200 mM with respect to NaCl and 80 mM with respect to Tris/HCl buffer, pH 7.4. (6) Sample identical with (1) except that it was 250 mM with respect to NaCl and 100 mM with respect to Tris/HCl buffer, pH 7.4. Samples (1)–(6) were incubated at 37°C for 2 h before application to the gel. (7) Sample identical with (1) except that it was dialysed, at room temperature, for 20 h against 4 mM-Tris/HCl/8 M-urea/2% (w/v) sodium dodecyl sulphate/20 mM-iodoacetamide, pH 7.4. (8) Unreduced human subcomponent C1q; sample identical with (1) except that it was 500 mM with respect to NaCl and heated at 100°C for 2 min before application to the gel.



EXPLANATION OF PLATE 2

Electrophoresis on 5.6% polyacrylamide gels, run in buffer containing 0.2% sodium dodecyl sulphate, of the isolated subunits of subcomponent C1q

The subunits of subcomponent C1q, which had apparent mol.wts. of 69000 and 54000, were eluted in 1.0ml of 0.5% sodium dodecyl sulphate from unstained 5.6% polyacrylamide gels run in the presence of sodium dodecyl sulphate and then re-run under the conditions described below. The apparent molecular weights of the reduced and alkylated A, B and C chains are taken from Reid *et al.* (1972). (1) Unreduced C–C dimer subunit of apparent mol.wt. 54000; sample loaded on the gel in 4M-urea, 1% (w/v) sodium dodecyl sulphate and 20mM-iodoacetamide. (2) Reduced C–C dimer subunit; sample identical with (1) except that it was reduced with 20mM-dithiothreitol before alkylation with iodoacetamide. (3) Unreduced C–C dimer subunit; sample identical with (1) except that it was made 500mM with respect to NaCl. (4) Unreduced A–B subunit of apparent mol.wt. 69000; sample loaded on the gel in 4M-urea, 1% (w/v) sodium dodecyl sulphate and 20mM-iodoacetamide. (5) Reduced A–B subunit; sample identical with (4) except that it was reduced with 20mM-dithiothreitol before alkylation with iodoacetamide. (6) Unreduced A–B subunit; sample identical with (4) except that it was made 500mM with respect to NaCl.

ing at 100°C for 2min gave results similar to those obtained when the samples were incubated at 37°C for 1h. When the two subunits were eluted from an unstained gel and re-run with and without addition of salt to the eluate, the 54000-apparent-mol.wt. subunit aggregated in high salt concentration to give higher-mol.wt. components, some of which did not enter the gel, whereas the behaviour of the 69000-apparent-mol.wt. subunit was not affected (Plate 2). The latter, on reduction, gave the A and B chains, whereas the former gave only the C chain (Plate 2), i.e. the subunits are an A–B dimer and a C–C dimer. Reduction and alkylation of the whole C1q subcomponent yielded equimolar amounts of the A, B and C chains, as reported previously [Reid *et al.* (1972) and Fig. 1(*c*)].

Behaviour of subcomponent C1q on elution from Sephadex G-200 run in 6M-guanidinium chloride, pH7.0

When subcomponent C1q was run on a column of Sephadex G-200 in 6M-guanidinium chloride, pH7.0, it was eluted partially as an aggregate, but the main peak emerged slightly ahead of the immunoglobulin G heavy chain at a volume equivalent to a protein of mol.wt. 49000–50000 when compared with other unreduced protein standards. Examination of the peaks by polyacrylamide-gel electrophoresis showed that the aggregate peak contained mainly high-molecular-weight impurities and that both the rising and the falling shoulders of the main peak each contained the A, B and C chains in equimolar proportions as judged by scanning of the gels. Thus the A–B dimer and C–C dimer cannot be separated by this technique and presumably have very similar molecular weights. The chemical data suggest that the molecular weight of the A–B dimer would be 49000 and that of the C–C dimer 46000, the difference between them being due to the higher carbohydrate content of the A–B dimer. These values suggest that the elution from Sephadex G-200 in 6M-guanidinium chloride gave a more reliable estimate of molecular weight, and that the discrepancy with that obtained by the polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate is due to the 10% carbohydrate content of the A–B dimer compared with 4% found in the C–C dimer, as glycoproteins have often been reported as giving anomalously high molecular weights by this method.

Discussion

Yonemasu & Stroud (1972) reported the presence of two subunits of subcomponent C1q from its behaviour on polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate and estimated their mol.wts. to be 60000 and 42000. They further showed that the apparently larger subunit gave, on reduction, two components equivalent to

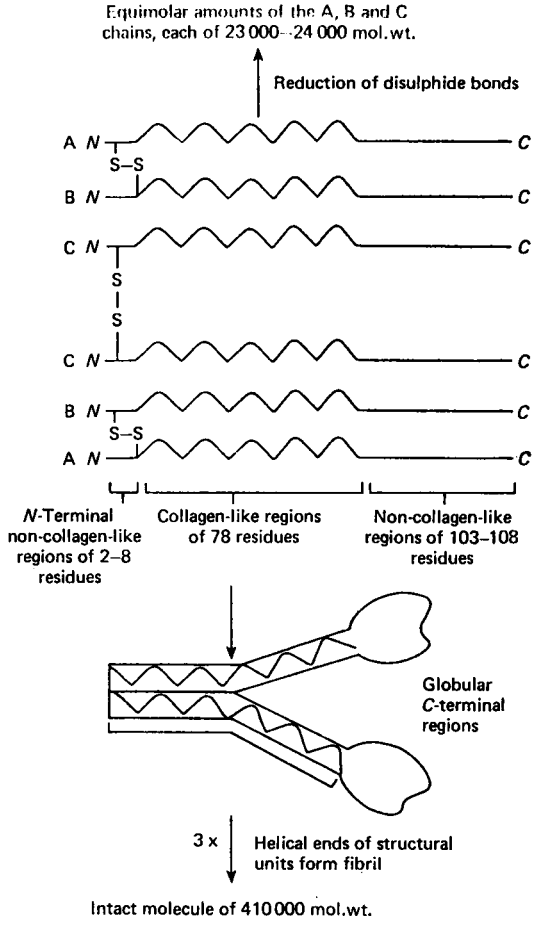


Fig. 2. *Suggested polypeptide-chain structure of human subcomponent C1q*

our A and B chains and that the smaller, on reduction, gave only the C chain. In our earlier studies (Reid *et al.*, 1972), because the samples were loaded in high salt concentration, the smaller subunit aggregated, only 5% being detectable in the expected position. The finding of this surprising aggregation of C–C dimer but not the A–B dimer in solutions of high ionic strength explains the different findings in the two laboratories. The ratio of A–B dimer to C–C dimer, by scanning, in the gel samples of lowest ionic strength was found to be 2:1, which is rather lower than the ratio of 2.9:1.0 reported by Yonemasu & Stroud (1972), presumably owing to a difference in salt content of the samples loaded.

In view of the results of elution rates from Sephadex G-200 in 6M-guanidinium chloride, we believe that the A–B and C–C dimers have similar molecular weights, of 50000 or less. This is in agreement with

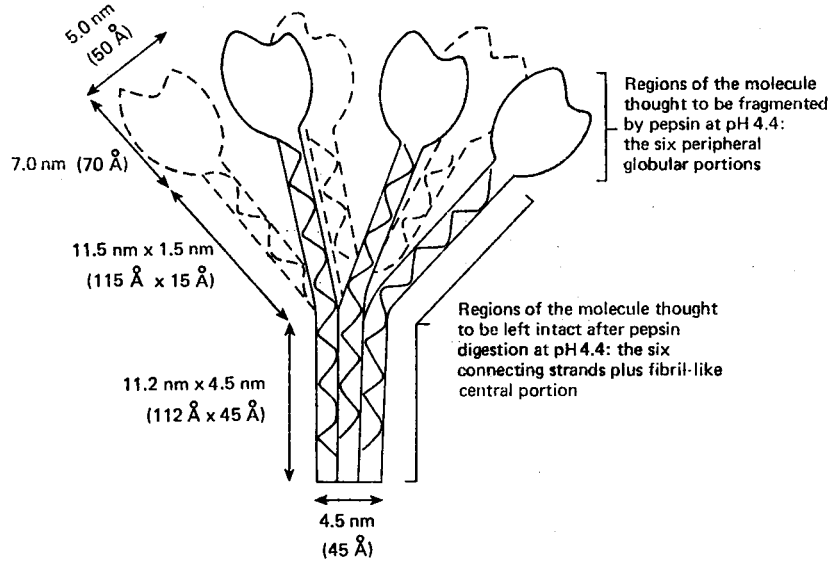


Fig. 3. *Proposed model of human subcomponent C1q*

A model of human subcomponent C1q is shown that is based on the electron-microscopy measurements (Shelton *et al.*, 1972; Knobel *et al.*, 1975), the results of the studies on the pepsin fragments of subcomponent C1q (Reid, 1976) and the assumption that the three collagen-like regions present in subcomponent C1q will form a triple helical collagen-type fibre. ——, portions of the molecule pointing towards the reader; – – – –, portions of the molecule pointing inward, away from the reader; ∼ and ∼, collagen-like regions of the molecule proposed to be in a triple helix. From the dimensions given, which are averages of those published by Shelton *et al.* (1972), the following comparison can be made. Length of collagen-like fibre (connecting strand)+length of fibril-like end portion = 11.5nm+11.2nm = 22.7nm (115Å+112Å = 227Å). Length of triple helix proposed from the sequence studies = 78×0.29nm = 22.6nm (78×2.9Å = 226Å).

chemical data reported previously (Reid *et al.*, 1972; Reid, 1974, 1976). It is suggested that in the whole subcomponent C1q molecule there are nine subunits of 46000–50000 mol.wt., six A–B dimers and three C–C dimers.

As discussed in previous papers (Reid, 1974, 1976), each of the three chains of subcomponent C1q contains 78 amino acid residues, near the *N*-terminal end, of typical collagen-like structure, with glycine in every third position and hydroxyproline and hydroxylysine often in the Y position of the repeating X-Y-Gly amino acid sequence. All collagen molecules contain three peptide chains of this structure; each chain is in a minor helix and the three chains are non-covalently associated together in a major helix to form a fibre of well-defined dimensions (Traub & Piez, 1971). As well as having the prerequisite for triple-helix formation of glycine as every third residue, the collagen-like sequences of 78 amino acid residues in the A, B and C chains have imino acid contents of 14, 23 and 26% respectively (Reid, 1976). These values are in the range found in vertebrate collagen partial sequences (18–27%; Dayhoff, 1972). Therefore, if collagen-like triple-helix structure is present in subcomponent C1q, it is probable that it will be composed of these 78 collagen-like sequences found in the A, B and C chains. They could yield a triple-helix-type fibre of dimensions 78×0.29nm (i.e. 22.6nm) long by 1.5nm diameter [78×29Å (i.e. 226Å) long by 15Å diameter] [0.29nm×1.5nm (2.9Å×15Å) being taken as the length and diameter respectively of the cross-section of a one-residue-long stretch of a collagen triple helix] (Traub & Piez, 1971). In view of the location of the interchain disulphide bond between two C chains (Reid, 1976), each triple-helix fibre would have to be joined to another fibre via the C-chain interchain bond (Fig. 2). The six fibres lined, in parallel, along their *N*-terminal halves could give a fibril of about 4.0–5.0nm (40–50Å) diameter and 11.3nm (113Å) long, which could correspond to the fibril-like central portion [4.5nm×11.2nm (45Å×112Å)] seen in the electron-microscopy studies (Fig. 3). The remaining halves of each of the six individual fibres [1.5nm×11.3nm (15Å×113Å) each], which were not involved in fibril formation, could represent the six connecting strands [1.5nm×11.5nm (15Å×115Å) each] seen in the electron-microscope studies (Fig. 4). Each connecting strand is joined to one of

the six peripheral globular portions [5.0nm×7.0nm (50Å×70Å) each], and each of these globular portions may therefore be composed of the *C*-terminal 103–108 residues of each of the A, B and C chains (Fig. 3). A feature of this proposed structure is that the entire molecule would be composed of three disulphide-bonded pairs of units, each unit being composed of an A, B and C chain (Fig. 2). The possibility that two A chains are in a triple-helix formation with one C chain and that two B chains are in a triple-helix formation with another C chain should also be considered. Further details of this proposed structure for subcomponent C1q have been published (Reid & Porter, 1975).

This remarkable structure appears to reconcile all the chemical and physical data and the known properties of collagen with the electron-microscope pictures of subcomponent C1q.

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