Chemical and hydrodynamic characterization of the human leucocyte receptor for complement subcomponent C1q

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A procedure for preparation of the receptor for complement subcomponent C1q from human tonsil lymphocytes and the monocytic cell line U937 was developed. The procedure is suitable for isolation of several hundred micrograms of the receptor, C1q-R, and has yielded sufficient material for chemical and hydrodynamic characterization. C1q-R from tonsil lymphocytes behaves identically with that from U937 cells. C1q-R has a monomer M_r of 56000, and is an acidic glycoprotein containing about 17% carbohydrate. The polypeptide chain length is estimated to be 416–448 amino acid residues, with two or three sites for *N*-linked glycosylation. Detergent-solubilized C1q-R exists as an elongated dimer ($f/f_0 = 1.8$), and does not bind a significant weight of detergent. The radioiodinated isolated receptor binds specifically and saturably to solid-phase C1q, but not to collagen, IgG, bovine serum albumin or complement component C3.

INTRODUCTION

C1q is a subcomponent of C1, the first component of the human complement system. *In vitro*, C1q has been shown to bind specifically to various types of cells through the collagenous (tail) region of C1q. Cell types suggested to have C1q receptor activity include B-lymphocytes, T-lymphocytes, B-lymphoblastoid cell lines such as Raji and Daudi, monocytes, polymorphonuclear leucocytes, endothelial cells, fibroblasts, U937 cells and platelets (Ghebrehiwet, 1987). The binding of C1q to cells *in vitro* has been reported to mediate antibody-dependent cell-mediated cytotoxicity (Ghebrehiwet & Müller-Eberhardt 1978), to enhance phagocytosis of C3b/iC3b-coated (Sorvillo *et al.*, 1986) or IgG-opsonized target particles (Boback *et al.*, 1987), to modulate synthesis of immunoglobulin by B-lymphocytes (Tenner *et al.*, 1987), to inhibit interleukin 1 production by B-lymphocytes (Habicht *et al.*, 1987) and to suppress the proliferation of transformed lymphoid cell lines (Ghebrehiwet *et al.*, 1987).

A protein species with C1q-binding activity of M_r 56000–80000 has been purified from appropriate cell lines by various workers using C1q-Sepharose affinity chromatography (Ghebrehiwet *et al.*, 1984; Erdei & Reid, 1988; Malhotra *et al.*, 1988), and this is termed C1q receptor (C1q-R).

Our studies with U937 cells and lymphocytes from human tonsils confirmed the finding that only a molecular species of apparent M_r 56000, as estimated by SDS/polyacrylamide-gel electrophoresis, binds consistently to C1q-Sepharose (Malhotra *et al.*, 1988). Ligand affinity methods for isolation of C1q-R were found convenient for small-scale preparation, but at larger scale non-specific binding of higher- M_r proteins to C1q-Sepharose was observed and the yield of C1q-R was low. Further analysis of the protein, purified by use of C1q-Sepharose, by ion-exchange chromatography indicated that the apparent single species of 56000 M_r ,

consisted of two protein components, acidic and basic, of almost identical mobility on SDS/polyacrylamide-gel electrophoresis (Malhotra *et al.*, 1988). Only the acidic species re-bound directly to C1q-Sepharose. To overcome problems associated with the purification of C1q-R by the use of C1q-Sepharose, and to eliminate the basic component, another high-yield purification procedure was developed. This procedure also overcomes the limited lifetime (in terms of re-use) of C1q-Sepharose. By using this procedure up to 400 μ g of highly purified C1q-R (> 95% pure) was obtained, which permitted us to characterize the receptor further.

MATERIALS AND METHODS

Commercial materials and buffers

Nonidet P40, Emulphogene BC720 (polyoxyethylene-10 tridecyl ether), soya-bean trypsin inhibitor (type 1-S), pepstatin A, 1,10-phenanthroline, ovalbumin, iodoacetamide and di-isopropyl phosphorofluoridate were all purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). Iodogen was obtained from Pierce and Warriner (Chester, U.K.). Tissue-culture medium was purchased from Flow Laboratories (Irvine, Ayrshire, Scotland, U.K.).

Phosphate-buffered saline was 8.2 mM- Na_2HPO_4 /1.5 mM- KH_2PO_4 buffer, pH 7.5, containing 139 mM-NaCl and 3 mM-KCl.

Lysis buffer was 10 mM-sodium phosphate buffer, pH 7.4, containing 0.15 mM- CaCl_2 , 0.5 mM- MgCl_2 , 1% (w/v) Nonidet P40, 100 μ g of soya bean trypsin inhibitor/ml, 5 mM-iodoacetamide, 2.5 mM-di-isopropyl phosphorofluoridate, 20 μ M-1,10-phenanthroline and 5 μ g of pepstatin/ml.

Cells

U937 cells, a monocytic cell line, were grown in 700 ml stirrer culture flasks in standard conditions (Malhotra &

Abbreviation used: C1q-R, C1q receptor.

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Sim, 1985) except that foetal-calf serum was replaced by 10 % (v/v) newborn-calf serum.

Lymphocytes were prepared from human tonsils (obtained from the E.N.T. Department, Radcliffe Infirmary, Oxford, U.K.) as described by Micklem *et al.* (1984).

Radioiodination of isolated C1q-R

Radioiodination was carried out by the Iodogen method (Fraker & Speck, 1978) with 1 mCi of Na¹²⁵I for radioiodination of 0.5 ml of protein solution (150 µg/ml) in 10 mM-sodium phosphate buffer, pH 7.4, containing 0.1 % (v/v) Emulphogene BC720 and 150 mM-NaCl. Excess free ¹²⁵I⁻ was removed by desalting on a PD-10 Sephadex G-25 (medium grade) gel-filtration column (Pharmacia, Milton Keynes, Bucks., U.K.) run in the same buffer. The specific radioactivity of the labelled C1q-R isolated as described here was about 570 000 d.p.m./µg of C1q-R.

Assay of binding of C1q-R to solid-phase C1q

Micro-titre plate (Sterlin, Hounslow, Middx., U.K.) wells were coated with 100 µl of C1q (10 µg/ml). C1q was kindly donated by Dr. K. B. M. Reid, M.R.C. Immunochimistry Unit, Oxford, U.K., and was purified by the method of Reid (1981). Control wells were coated with non-immune human IgG (10 µg/ml), collagen type IV (soluble) (10 µg/ml; Sigma Chemical Co.), bovine serum albumin (10 µg/ml; Sigma Chemical Co.) or complement component C3 (10 µg/ml; prepared by the method of Sim *et al.*, 1981). The buffer used for coating wells was 10 mM-potassium phosphate buffer, pH 7.4, containing 5.0 mM-EDTA and 100 mM-KCl. Non-specific binding sites were blocked with 300 µl of bovine serum albumin (5 mg/ml in the same buffer). Serial dilutions of ¹²⁵I-labelled C1q-R (128 000 d.p.m. to 4000 d.p.m.) in 10 mM-potassium phosphate buffer, pH 7.4, were added to coated wells and the plate was incubated for 2 h at ambient temperature (18–22 °C). After three washes with 300 µl portions of 10 mM-potassium phosphate buffer, pH 7.4, bound ¹²⁵I-labelled C1q-R was solubilized and removed with 400 µl of 4 M-NaOH. Radioactivity obtained from each individual well was measured in an LKB 1275 Minigamma counter.

SDS/polyacrylamide-gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out as described by Laemmli (1970). Samples were prepared (reduced or alkylated) as described by Fairbanks *et al.* (1971). Proteins on gels were detected with Coomassie Brilliant Blue (Sigma Chemical Co.) and/or by silver staining (National Diagnostic, Aylesbury, Bucks., U.K.).

Purification of C1q-R

Lymphocytes separated from 22–30 pairs of human tonsils (approx. 1×10^{11} – 3×10^{11} cells) were washed with three 100 ml portions of phosphate-buffered saline. The cells were resuspended in lysis buffer (240 ml), left for 1 h at 4 °C, then centrifuged at 27 000 g for 40 min at 4 °C. In order to monitor the purification of C1q-R in the large-scale preparation, highly purified C1q-R derived from a small-scale ligand-affinity-based preparation (Malhotra *et al.*, 1988) was radioiodinated, and was added as 'tracer' (approx. 570 000 d.p.m., in 100 µl) to the cell lysate. The soluble extract was then pre-cleared by incubation with 20 ml of non-immune human

IgG-Sepharose (10 mg of IgG/ml of packed Sepharose) and then loaded on to a column (12 cm × 2 cm diam.) of DEAE-Sepharcel (Pharmacia) equilibrated in 10 mM-sodium phosphate buffer, pH 7.4, containing 0.1 % Emulphogene BC720 and 0.2 mM-EDTA. The column was washed with 7 column volumes of starting buffer, and C1q-R was eluted with a linear gradient (total volume 400 ml) of 0–500 mM-NaCl in starting buffer.

The pool containing C1q-R from DEAE-Sepharcel was dialysed against 10 mM-sodium phosphate buffer, pH 7.4, containing 0.1 % Emulphogene BC720, then fractionated on a Mono Q f.p.l.c. column (1 ml) (Pharmacia) equilibrated in the same buffer. The column was washed with 10 ml of starting buffer, and C1q-R was eluted with a linear gradient (40 ml) of 0–1 M-NaCl in starting buffer. C1q-R was eluted at approx. 470 mM-NaCl. The pool containing C1q-R was concentrated 10-fold by partial freeze-drying, and the concentrated sample was applied to a Superose-12 (Pharmacia) f.p.l.c. gel-filtration column (300 mm × 10 mm diam.). Gel filtration was carried out in 50 mM sodium phosphate buffer, pH 7.4, containing 2 mM-EDTA, 150 mM-NaCl and 0.1 % (v/v) Emulphogene BC720. The final purified material was stored at 4 °C and appeared to be stable for about 6–10 weeks without addition of proteinase inhibitors.

Sucrose-density-gradient centrifugation

Linear sucrose-density gradients (13 ml) of 5–40 % (w/v) sucrose in 10 mM-sodium phosphate buffer, pH 7.4, with or without detergent [0.1 % (v/v) Emulphogene BC720] were prepared by the method of Martin & Ames (1961). Samples of purified C1q-R (500 µl; 10–15 µg of protein) or ¹²⁵I-labelled C1q-R (500 µl; 850 000 d.p.m.) in 10 mM-sodium phosphate buffer, with or without 0.1 % Emulphogene BC720, were loaded on to the gradients and centrifuged in a Beckman SW40 Ti rotor at 37 000 rev./min (264 000 g) for 16–48 h at 4 °C. Gradients were fractionated into 20–24 tubes by peristaltic pumping from the base of the gradient. Gradient fractions were analysed by SDS/polyacrylamide-gel electrophoresis, by reading A_{280} and by measuring radioactivity. The $s_{20,w}$ value for C1q-R was estimated by measuring its mobility on the gradient relative to those of standard proteins (Martin & Ames, 1961). Standard proteins were run in the same gradient as C1q-R, and in separate gradients, and consisted of human IgG (7.3 S), bovine liver catalase (11.2 S), bovine serum albumin (4.7 S), soya-bean trypsin inhibitor (2.3 S), hen's-egg lysozyme (1.9 S) and cytochrome *c* (1.7 S). Sedimentation coefficients for these proteins were taken from Smith (1970).

Determination of Stokes radius

Elution of C1q-R, relative to standards, was studied on a Superose-12 f.p.l.c. gel-filtration column (300 mm × 10 mm diam.). Void volume (7.3 ml) of the column was calculated from the elution volume of human IgG aggregates. Human IgG (monomer), bovine serum albumin and soya-bean trypsin inhibitor (respective Stokes radii 5.4, 3.6 and 2.4 nm; Smith, 1970) were used to calibrate the column. Gel filtration was carried out in 50 mM-sodium phosphate buffer, pH 7.4, containing 150 mM-NaCl and 5 mM-EDTA, with or without detergent [0.1 % (v/v) Emulphogene BC720]. The Stokes radius of C1q-R was calculated as described by Ackers (1964).

Carbohydrate estimation

An estimate of the carbohydrate content of isolated C1q-R was made by the SDS/polyacrylamide-gel-electrophoresis method of Segrest & Jackson (1970). Migration of C1q-R and a positive control, the highly glycosylated protein C1 inhibitor, was studied in gels of different percentage acrylamide (6–12%), and mobility was calculated relative to those of standards that were non-glycosylated or of low carbohydrate content. C1 inhibitor was isolated by the method of Sim & Reboul (1981).

Amino acid and amino sugar analysis

C1q-R (15 µg) in 10 mM-sodium phosphate buffer, pH 7.4, containing 150 mM-NaCl and 2 mM-EDTA (500 µl) was denatured by addition of 2 ml of 7.5 M-guanidinium chloride/0.125 M-Tris/HCl buffer, pH 8.0, and reduced by addition of dithiothreitol to a final concentration of 6.5 mM. The sample was incubated for 1 h at 37 °C, then 4-vinylpyridine (Aldrich Chemical Co., Gillingham, Dorset, U.K.) was added to a final concentration of 88 mM to block free cysteine residues. The sample was further incubated for 1 h at 37 °C, then dialysed exhaustively against 10 mM-NH₃ in water, and freeze-dried. The sample was either (a) hydrolysed with 6 M-HCl for 24 h at 110 °C or (b) hydrolysed with 3 M-toluene-*p*-sulphonic acid at 100 °C for 24 h (Allen & Neuburger, 1975), and amino acid composition (a) or amino sugar content (b) was analysed by using an LKB 4400 amino acid analyser.

Another sample of C1q-R was denatured with the guanidinium chloride-containing buffer as above, but was not reduced. It was dialysed against 10 mM-NH₃ in water, freeze-dried and subjected to performic acid oxidation (Hirs, 1956). After a further freeze-drying the sample was hydrolysed in 6 M-HCl as above, and the hydrolysate was treated with phenyl isothiocyanate (Heinrikson & Meredith, 1984). Phenyl isothiocyanate derivatives of amino acids were analysed by h.p.l.c. with the Waters Pico-Tag system (Millipore, Watford, Herts., U.K.). This permitted measurement of cysteine as cysteic acid.

RESULTS

Purification of C1q-R

The profile of elution of detergent-soluble tonsil lymphocyte proteins from DEAE-Sephacel is shown in Fig. 1. Fractions eluted at 350–400 mM-NaCl, indicated by a horizontal bar in Fig. 1, were found to be co-eluted with the ¹²⁵I-labelled 'tracer' C1q-R. The elution position of C1q-R was confirmed by analysing individual fractions by SDS/polyacrylamide-gel electrophoresis. A 56000-*M_r* band present in these fractions has the same mobility as ¹²⁵I-labelled C1q-R. Other peaks of radioactivity observed on Fig. 1 correspond to radiolabelled detergent (impurities in Emulphogene BC720: fractions 1–110) or to breakdown products (as assessed by mobility on SDS/polyacrylamide-gel electrophoresis) of ¹²⁵I-labelled C1q-R. The basic contaminant of apparent *M_r* 56000 noted in C1q-R preparations made by C1q-Sepharose affinity chromatography (Malhotra *et al.*, 1988) is eliminated at this stage, as it does not bind to DEAE-Sephacel.

The pool containing C1q-R was further purified by

Fig. 1. DEAE-Sephacel chromatography

Details are given in the Materials and methods section. Bound protein was eluted on a linear gradient of 0–500 mM-NaCl (—); 3.5 ml fractions were collected. Radioactivity (d.p.m.) (.....) and *A*₂₈₀ (—) were measured. The horizontal bar shows fractions containing C1q-R that were pooled for further purification. Earlier-eluted radioactive material results partly from the degradation of the tracer ¹²⁵I-labelled C1q-R and partly from the presence of radioactive detergent. The high *A*₂₈₀ in approx. fractions 1–80 is due to elution of the detergent Nonidet P40 used for solubilization.

Fig. 2. Mono Q f.p.l.c. chromatography

Details are given in the Materials and methods section. —, Linear gradient of 0–1 M-NaCl; 0.5 ml fractions were collected at a flow rate of 1 ml/min. Radioactivity (d.p.m.) (.....) was measured and *A*₂₈₀ (—) was continuously monitored.

Mono-Q f.p.l.c. (Fig. 2) and Superose-12 f.p.l.c. (Fig. 3), as described in the Materials and methods section. C1q-R is eluted from Mono Q at 440–500 mM-NaCl (Fig. 2). The elution position of C1q-R was assessed from the appearance of the characteristic 56000-*M_r* band in SDS/polyacrylamide-gel electrophoresis and from co-elution with the ¹²⁵I-labelled C1q-R 'tracer'. Variable traces of contaminants of apparent *M_r* 30000 and 85000 (in non-reducing conditions) remain, and these were removed by gel filtration (Fig. 3).

The appearance of the final preparation on SDS/polyacrylamide-gel electrophoresis is shown in Fig. 4. In non-reducing conditions (Fig. 4, track 1) C1q-R appears as a 53000-*M_r* species (assessed relative to reduced standards). A trace contaminant of slightly higher *M_r* is sometimes visible. On reduction (Fig. 4, track 3) C1q-R is of apparent *M_r* 56000. Several minor

bands are visible in the reduced material. These are likely to represent fragments from proteolytic cleavage of C1q-R.

The yield of total protein at each stage of purification from tonsil lymphocytes is shown in Table 1, and is compared with a preparation from U937 cells (10^{11} cells). C1q-R from U937 cells behaved identically with that from tonsil lymphocytes throughout purification and analysis. There is no satisfactory assay for measuring C1q-R specific activity or total activity, and so the efficiency of the preparation (i.e. the proportion of total C1q-R recovered) has not been determined. However, in terms of total protein, C1q-R has been purified 640-fold from the tonsil lymphocyte extract and 850-fold from the U937-cell extract.

Activity of C1q-R

To confirm that the purified material has appropriate activity, binding of ^{125}I -labelled C1q-R to C1q immobilized on micro-titre plates was tested as described

Fig. 3. Superose-12 gel-filtration chromatography

Details are given in the Materials and methods section. Fractions (0.5 ml) were collected at a flow rate of 0.3 ml/min. A_{280} (—) was monitored continuously and ^{125}I radioactivity (d.p.m.) (.....) was measured in each fraction. Elution positions of standards [1 mg each of human IgG, bovine serum albumin (BSA) and soya-bean trypsin inhibitor (SBTI)], used in determining the Stokes radius of C1q-R, are indicated.

in the Materials and methods section. Significant and saturable binding to C1q was observed (Fig. 5), but binding to human IgG, collagen, bovine serum albumin and C3 was negligible.

Chemical analysis of C1q-R

The amino acid composition of isolated C1q-R is shown in Table 2, and is compared with a previous analysis published by Ghebrehiwet *et al.* (1984). The

Fig. 4. Purified C1q-R after Superose-12 gel-filtration chromatography analysed by SDS/polyacrylamide-gel electrophoresis on 10% (w/v) acrylamide gels and detected by silver staining

Track 1, C1q-R (non-reduced); track 2, reduced standards [a, phosphorylase *b* (M_r 92000); b, catalase (M_r 60000); c, ovalbumin (M_r 42000); d, carboxypeptidase (M_r 25000)]; track 3, C1q-R (reduced).

Table 1. Protein yields during purification of C1q-R from tonsil lymphocytes and U937 cells

Experimental details are given in the Materials and methods section. Protein concentration of C1q-R-containing pools was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Purification stage	Total protein (mg)	
	U937-cell lysate	Tonsil cell lysate
Detergent-soluble extract of cells	145	235
C1q-R pool after IgG-Sepharose chromatography	92.5	150
C1q-R pool after DEAE-Sepharose chromatography	3.78	2.9
C1q-R pool after Mono Q f.p.l.c.	0.6	0.6
C1q-R pool after Superose-12 gel filtration	0.17	0.37

Fig. 5. Binding activity of C1q-R to solid-phase ligands

Binding of ^{125}I -labelled C1q-R ($2\text{ }\mu\text{g/ml}$) to solid-phase C1q (○), IgG (●) and bovine serum albumin (□) is shown. Binding of ^{125}I -labelled C1q-R to solid-phase collagen and C3 was similar to that of IgG and bovine serum albumin. Details are given in the Materials and methods section.

composition is remarkable for the high content of aspartic acid/asparagine and glutamic acid/glutamine. The acidic nature of the protein, as seen on DEAE-Sephacel chromatography, indicates that a high proportion of these residues is non-amidated. The composition reported here has features in common with that of Ghebrehwet *et al.* (1984), in that aspartic acid/asparagine, glutamic acid/glutamine and glycine are the major components in both analyses. However, there is wide disagreement for some residues (e.g. serine, proline, isoleucine and leucine). Further purification of affinity-purified C1q-R (i.e. material obtained by a single-step purification on C1q-Sepharose) has shown that it contains a basic contaminant, which co-runs with C1q-R on SDS/polyacrylamide-gel electrophoresis (Malhotra *et al.*, 1988). This contaminant may have been present in the affinity-purified material analysed by Ghebrehwet *et al.* (1984).

Hexosamine analysis (Table 2) indicates that there are approx. 2.3 glucosamine residues per 100 amino acid residues. The galactosamine value is low, in contrast with that reported by Ghebrehwet *et al.* (1984).

The SDS/polyacrylamide-gel-electrophoresis method of Segrest & Jackson (1970) was used to obtain an estimate of the overall percentage carbohydrate in C1q-R. This procedure involves measurement of migration of C1q-R in gels of different percentage acrylamide, observing an asymptotic minimal M_r (Fig. 6) and calculating percentage carbohydrate from standard curves (Fig. 3 and Fig. 4 of Segrest & Jackson, 1970). By this method, the percentage carbohydrate of the positive control, C1

Table 2. Amino acid and amino sugar composition of C1q-R

The analysis of C1q-R prepared here (A) is compared with an analysis (B) published by Ghebrehwet *et al.* (1984). Cysteine was determined as cysteic acid (A). Abbreviation: N.D., not determined.

Amino acid	Composition (residues/100 amino acid residues)	
	A	B
Asx	14.7	9.4
Thr	3.6	5.1
Ser	6.2	8.3
Glx	16.8	10.3
Pro	6.2	8.1
Gly	14.5	19.1
Ala	4.5	7.5
Val	4.5	4.2
Cys	2.7	0
Met	0.9	0
Ile	4.1	2.6
Leu	5.2	7.8
Tyr	2.5	3.1
Phe	3.6	3.5
His	1.3	1.7
Lys	6.3	5.5
Arg	2.3	3.9
Trp	N.D.	N.D.
Total	99.9	100.1
Glucosamine	2.3	2.3
Galactosamine	1.0	7.5

inhibitor, was found to be $37 \pm 4\%$, in good agreement with the accepted range (Sim & Reboul, 1981). C1q-R was found to contain $17 \pm 3\%$ carbohydrate. On the basis that the monomer M_r of C1q-R is 56000, the C1q-R monomer contains 8000–11000 M_r of carbohydrate. This implies a polypeptide chain length of 416–448 amino acid residues (polypeptide M_r 44800–48200), with about ten glucosamine residues per polypeptide chain. The glucosamine content and overall percentage carbohydrate are consistent with the presence of two or three *N*-linked complex oligosaccharide units per polypeptide chain (Wagh & Bahl, 1981; Hatton *et al.*, 1983). The low galactosamine content indicates that zero to four *O*-linked sugar residues may be present.

M_r of C1q-R in non-denaturing conditions

The Stokes radius of C1q-R was found to be $5.7 \pm 0.2\text{ nm}$, by using the procedures described in the Materials and methods section. Elution positions of C1q-R and protein standards are indicated in Fig. 3. The presence or absence of detergents (0.1% Emulphogene BC720) made no difference to the behaviour of C1q-R in gel filtration.

The sedimentation coefficient for C1q-R was found to be $4.8 \pm 0.2\text{ S}$ by using the method of Martin & Ames (1961). Both C1q-R and ^{125}I -labelled C1q-R migrated as a single symmetrical peak. Again the presence or absence of detergent did not affect the mobility of C1q-R, except that slight aggregation ($< 10\%$) was observed in the absence of detergent. The behaviour of the protein with and without detergent in both gel filtration and

Fig. 6. Variable electrophoretic migration of C1q-R in SDS/polyacrylamide gels of different percentage acrylamide

C1q-R (○) and a positive control, C1 inhibitor (●), were run, reduced, on gels of different acrylamide content (5–12%) and their apparent M_r values were calculated relative to standards as described in the Materials and methods section.

centrifugation indicates that it does not bind a significant weight of detergent. The slight aggregation on centrifugation in the absence of detergent may, however, indicate that a few molecules of detergent may remain bound to the solubilized receptor and may influence aggregation. Since, therefore, there is little or no detergent in the complex, the Stokes radius and sedimentation coefficient of C1q-R can be used to calculate the glycoprotein M_r . M_r was calculated as summarized by Siegel & Monty (1966) from the equation:

$$M_r = (6\pi\eta Ns)/(1 - \bar{v}\rho)$$

where a is the Stokes radius, s is sedimentation coefficient, N is Avogadro's number, $\bar{v}$ is the partial specific volume (taken to be 0.73, an average value for proteins and glycoproteins of moderate carbohydrate content), ρ is the density of water at 20 °C and η is the viscosity of water at 20 °C.

The calculated M_r is 115000 ± 7000 , which in comparison with the SDS/polyacrylamide-gel-electrophoresis-derived value of 56000 indicates that the solubilized C1q-R is a dimer.

The friction coefficient f/f_0 was calculated as 1.8 from the equation:

$$f/f_0 = a/[(3\bar{v}M_r)/(4\pi N)]^{1/2}$$

Comparing this value with those of other non-globular proteins, e.g. IgG ($f/f_0 = 1.5$; Smith, 1970) or complement factor H ($f/f_0 = 2.1$; Sim & DiScipio, 1982) it is clear that the C1q-R dimer is highly elongated.

DISCUSSION

The isolation procedure for C1q-R described here provides sufficient material for a more detailed analysis of C1q-R than has been possible before. The material isolated is about 95% pure, and has a monomer M_r of 56000. As shown previously (Malhotra *et al.*, 1988), it is a cell-surface protein, as assessed by incorporation of ^{125}I during radioiodination of intact U937 cells. SDS/polyacrylamide-gel electrophoresis of the unfractionated detergent-solubilized extract of U937 cells or tonsil lymphocytes (results not shown) indicates that the 56000- M_r species is not a major cell component (< 2%); similarly on SDS/polyacrylamide-gel analysis of surface-radioiodinated U937 cells the 56000- M_r species is not a major radiolabelled band (< 5%). However, in both cases there are several components of similar apparent M_r in the cell extracts. The quantity purified from tonsil cells or U937 cells (Table 1) represents about 10^4 molecules per cell, although the percentage yield of C1q-R has not been measured. Several studies of binding of ^{125}I -labelled C1q to cells, summarized by Ghebrehwet (1989), have suggested very high numbers of C1q receptors on various cell types (10^5 up to 3×10^6). In tests of this type it is difficult to assess the extent of non-specific binding and of internalization of ligand, and considerable overestimates may arise. Values below 10^5 C1q-R molecules per leucocyte may be more realistic, assuming, conservatively, that our purification has only a 10% yield.

Isolation procedures for C1q-R described previously rely, generally, on affinity chromatography on C1q-Sepharose without further chromatographic steps. A contaminant basic protein that co-migrates with C1q-R on SDS/polyacrylamide-gel electrophoresis has been identified in preparations of this type (Malhotra *et al.*, 1988). The presence of variable amounts of this contaminant in C1q-R may have led to some confusion in earlier reports on this receptor. Ghebrehwet (1987), for example, reports that C1q-R sometimes migrates as two closely spaced bands on SDS/polyacrylamide-gel electrophoresis, and is heterogeneous in sucrose-density-gradient centrifugation. Erdei & Reid (1988) also show C1q-R, prepared by a single affinity step, migrating as a diffuse band on SDS/polyacrylamide-gel electrophoresis, and with evident heterogeneity in gel filtration. Ghebrehwet (1989) discusses such inhomogeneities further, and concludes that contaminants are likely to be present.

Our larger-scale preparative method eliminates the ligand affinity step and relies on the acidic nature of C1q-R to provide a high initial degree of purification by ion-exchange, with removal of minor impurities by gel filtration. The isolated C1q-R is apparently homogeneous in gel filtration (Fig. 3) and on sucrose-density-gradient centrifugation (results not shown), but minor contamination and proteolysis are still visible on SDS/polyacrylamide-gel electrophoresis at high loading (Fig. 4).

There has also been some confusion over the monomer M_r of C1q-R as assessed by SDS/polyacrylamide-gel electrophoresis. Erdei & Reid (1988), using the same gel system and standards as described here, but using 7.5% (w/v) acrylamide gels, estimated C1q-R as about 60 000 M_r (non-reduced) and 65 000–70 000 M_r (reduced). As seen from Fig. 6, this is consistent with our estimates of 53 000 (non-reduced) or 56 000 (reduced) on 10% gels. The lower the percentage acrylamide, the greater the overestimate of M_r of the C1q-R glycoprotein when compared with standards with little or no carbohydrate. Ghebrehiwet *et al.* (1984) and Ghebrehiwet (1987) give much higher values of 70 000 and 80 000–90 000, stating in the latter paper that the higher value is for the reduced protein, and in the former paper that the higher value is for the non-reduced protein. In at least one case (Ghebrehiwet *et al.*, 1984) these values were found in a gradient gel system. Given that it is not possible to estimate the percentage acrylamide through which the C1q-R migrated, it is possible that these high values are still consistent with those reported here and by Erdei & Reid (1988). The problem of variation in reported M_r values is likely to result merely from differences in the SDS/polyacrylamide gel system used.

The amino acid composition of C1q-R is unusual in its high content of aspartic acid/asparagine, glutamic acid/glutamine and glycine. These three residues account for 45% of total amino acid residues. The hexosamine analysis is consistent with the estimate of total carbohydrate made by the method of Segrest & Jackson (1970). There is no indication from the chemical or M_r analyses that C1q-R contains a proteoglycan moiety, as suggested by Ghebrehiwet *et al.* (1984). Detergent-solubilized C1q-R exists as a dimer of, presumably identical, non-covalently linked 56 000 M_r subunits. The behaviour of C1q-R does not alter when excess detergent is removed by gel filtration or centrifugation, and C1q-R appears to be stable in solution in the absence of detergent, in contrast with an earlier statement (Ghebrehiwet, 1987). Solubilized C1q-R is reported to be able to precipitate C1q in double-radial immunodiffusion assays (Ghebrehiwet, 1987), and this is consistent with it being a dimer, having two binding sites for C1q. The precipitation phenomenon also implies that C1q has more than one binding site for C1q-R, allowing cross-linked aggregates to form and be precipitated.

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