The Binding Protein for Globular Heads of Complement C1q, gC1qR
FUNCTIONAL EXPRESSION AND CHARACTERIZATION AS A NOVEL VITRONECTIN BINDING FACTOR*

(Received for publication, January 22, 1996, and in revised form, July 17, 1996)

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A binding protein for the globular head domains of complement component C1q, designated gC1qR, recently described to be present on vascular and blood cells (Ghebrehiwet, B., Lim, B.-L., Peerschke, E. I. B., Willis, A. C., and Reid, K. B. M. (1994) J. Exp. Med. 179, 1809–1821) was expressed in recombinant form in bacteria to investigate its functional and structural properties. The recombinant gC1qR was found to be functional because tetramerization of the 24.3-kDa polypeptide occurred as described for the native protein, and the binding of the ligand C1q by recombinant gC1qR was indistinguishable from binding shown by gC1qR isolated from Raji cells. Recombinant gC1qR immobilized to microspheres was used to search for additional binding proteins unrelated to C1q. Surprisingly, it was found that vitronectin or complexes containing vitronectin were retained from plasma or serum, and subsequent analysis revealed the specific binding of the ternary vitronectin-thrombin-antithrombin complex to gC1qR. Because the thrombin-antithrombin complex was unable to interact with gC1qR, direct binding with vitronectin was investigated in a purified system. The heparin binding multimeric form of vitronectin but not the plasma form of vitronectin was found to bind specifically to gC1qR isolated from Raji cell membrane as well as to recombinant gC1qR. This interaction was saturable ($K_D = 20$ nM) and inhibitable by glycosaminoglycans such as heparin but not by chondroitin sulfate. C1q and vitronectin did not compete with each other for binding to gC1qR, and both ligands seem to interact with different parts of the gC1qR because a truncated version of recombinant gC1qR lacking the N-terminal 22-amino acid portion hardly interacted with vitronectin but bound C1q as well as the intact gC1qR. These findings establish gC1qR as a novel vitronectin-binding protein that may participate in the clearance of vitronectin-containing complexes or opsonized particles or cooperate with vitronectin in the inhibition of complement-mediated cytolysis.

Various receptor systems are present on vascular cells and circulating blood cells that are not only responsible for early recognition processes in humoral defense mechanisms, such as complement or blood coagulation, but also serve to eliminate “spent” molecules of these cascade systems. In particular, various types of complement receptors that belong to different classes of super gene families (1) have been characterized that serve to concentrate the initiation, regulation, and control of complement activation to cell surfaces of invading particles (2, 3). In particular, two types of cell surface proteins that bind to the complement subcomponent C1q have been described, and they are distinguished by their ligand recognition specificity. One type (cC1q receptor) binds to the collagenous portion of C1q (4, 5), whereas the other type of C1q-binding protein (gC1qR) interacts with the globular heads of C1q (6, 7). The gC1qR was initially purified from a Raji cell membrane fraction, and use was made of partial protein sequence data to clone the corresponding cDNA from a B-cell cDNA library. The cDNA encoded a polypeptide of 282 residues, as judged by the position of the first methionine, whereas the N-terminal sequence of the mature protein, isolated from Raji cells, began with residue 74 (Leu) of the prepro-protein. By using a polyclonal antibody against gC1qR, it has been shown to be present on the surface as well as in the cytosolic fraction of human neutrophils (7).

To expand our knowledge about the structural and functional properties of this C1q-binding protein, bacterial expression of recombinant forms of gC1qR was performed, and these forms were used for ligand binding assays. In addition to the expected ligand C1q, it was found that vitronectin-containing complexes, particularly present in serum, were bound by gC1qR, and the binding characteristics were established in a purified system. Together with heparin binding forms of vitronectin, gC1qR may be part of a novel mechanism that may serve as a direct inhibitor of the terminal phase of the complement cascade, but it could also act as a scavenger or opsonizing complex in the clearance/phagocytosis phase. Thus, the identification of the vitronectin-gC1qR relationship provides a novel link between the initial events and the terminal phase of the complement cascade in immune defense.

EXPERIMENTAL PROCEDURES

Materials—The following chemicals and reagents were purchased from the sources indicated: isopropyl $\beta$-D-thiogalactoside and protrombin (Novo Biochem, Nottingham, United Kingdom); $N$-hydroxy succinimido-biotin and sulfo succinimidyl 2-(biotinamido)ethyl-1,3-dithiopropioni-
eluted with 0.1M glycine HCl, pH 2.5, and 0.5-ml fractions were collected into tubes containing 50 ml of 150 mM NaCl, 0.05% (w/v) NaN₃, and 0.05% (v/v) Tween 20, pH 7.5) and the supernatant was loaded onto the preclear column and then onto the recombinant gC1qR-agarose column. All washing and elution steps were done accordingly at 4°C. The identity of the eluted proteins was determined by N-terminal protein sequencing and Western blotting.

**RESULTS**

**Expression and Purification of Recombinant gC1qR**—The different stages of expression and purification of the recombinant gC1qR protein, including the binding of the glutathione S-transferase fusion product to glutathione-Sepharose, digestion of thrombin and elution, and separation on Mono-Q followed by salt gradient elution, were followed by SDS-polyacrylamide gel electrophoresis. The nature of the different protein pools at each step is documented in Fig. 1. By N-terminal protein sequencing, the protein bands in lanes 7 and 8 were shown to be glutathione S-transferase and the recombinant gC1qR, respectively. On these denatured gels the recombinant protein (24.3 kDa) exhibited a lower mobility than its fusion partner, glutathione S-transferase (26 kDa). The yield of the recombinant protein was approximately 30 mg per liter of culture broth.

The amino acid sequences of the polypeptides derived from the cDNA and of the recombinant protein are given elsewhere (6). The native protein isolated from Raji cell membrane
starts with residue 74 of the putative translated sequence, whereas the recombinant protein contains 5 additional amino acid residues (Gly-Ser-Pro-Met-Ala) at the N terminus that are derived from the vector sequence. Upon gel filtration, the recombinant gC1qR as well as the gC1qΔRN (obtained under identical conditions) were discernible as single peaks at a position of apparent molecular mass of 97.2 and 88 kDa, respectively, indicating the formation of tetramers under non-denaturing conditions (Fig. 2). The tetrameric structure of gC1qR was also found after sucrose gradient ultracentrifugation and cross-linking studies followed by SDS-polyacrylamide gel electrophoresis (data not shown).

Recombinant gC1qR Recognizes C1q—At physiological ionic strength, the recombinant full-length protein binds to immobilized C1q, even at the low coating concentration of 10 ng/well (Fig. 3). Binding of gC1qR and ligand was mostly mediated by ionic interactions because 250 mM NaCl reduced specific binding to 50% of control, and 90% inhibition was observed in the presence of 600 mM NaCl. At these salt concentrations, the immobilized C1q was not removed from the microtiter plate. These findings indicate that the functional features of the recombinant gC1qR correlate with the known properties of the Raji cell membrane-derived protein.

Binding of Plasma and Serum Proteins to Immobilized gC1qR—To identify possible ligands for gC1qR in the circulation, serum and plasma, respectively, were passed over a gC1qR affinity column, and after extensive washing, bound proteins were eluted by high salt solution and subsequently identified by SDS-polyacrylamide gel electrophoresis (data not shown).

Vitronectin Interaction with C1q-binding Protein gC1qR

The various stages in the purification of the recombinant gC1qR fusion protein were analyzed by SDS-polyacrylamide gel electrophoresis on 15% (w/v) gels under reducing conditions: total cell lysate (lane 2); cell extracts before loading onto the glutathione affinity column (lane 3); flow-through of the affinity column (lane 4); elution of recombinant gC1qR fusion protein by reduced glutathione (lane 5); fusion protein digested by thrombin (lane 6); material from the thrombin digest that did not bind to the Mono-Q column (lane 7); and the recombinant gC1qR eluted by 0.6 M NaCl from the Mono-Q column (lane 8). Molecular weight markers (in thousands) in lanes 1 and 9 are indicated along the margins.

FIG. 1. Isolation of the gC1qR fusion protein. The various stages in the purification of the recombinant gC1qR fusion protein were analyzed by SDS-polyacrylamide gel electrophoresis on 15% (w/v) gels under reducing conditions: total cell lysate (lane 2); cell extracts before loading onto the glutathione affinity column (lane 3); flow-through of the affinity column (lane 4); elution of recombinant gC1qR fusion protein by reduced glutathione (lane 5); fusion protein digested by thrombin (lane 6); material from the thrombin digest that did not bind to the Mono-Q column (lane 7); and the recombinant gC1qR eluted by 0.6 M NaCl from the Mono-Q column (lane 8). Molecular weight markers (in thousands) in lanes 1 and 9 are indicated along the margins.

FIG. 2. Gel filtration of recombinant gC1qR. Marker proteins as indicated and recombinant gC1qR as well as the deletion mutant gC1qΔRN were analyzed by size-exclusion chromatography on a Superose-12 column, and the position of marker proteins (cytochrome c, soybean trypsin inhibitor (SBTI), ovalbumin, and bovine IgG) are indicated in the semi-logarithmic plot. Both forms of recombinant gC1qR elute as tetramers with physiological ionic strength buffer.

FIG. 3. Binding of recombinant gC1qR to immobilized C1q. A, biotinylated gC1qR was bound to microtiter wells coated with increasing amounts of C1q as indicated, and binding was detected by streptavidin-alkaline phosphatase reaction (A). No binding was observed to BSA (Δ) or when C1q coating was omitted (○). B, binding of biotinylated gC1qR to immobilized C1q was followed at various NaCl concentrations as indicated (○). No C1q was released by high salt concentrations as probed by direct antibody binding (△). The data represent the mean of duplicates of a typical experiment (<5% variation).

Theoretical derived from plasma, whereas vitronectin-positive material was recognized in both cases (Figs. 4 and 5). Indeed, both vitronectin typical bands in reduced samples were discernible after Western blot analysis. Although plasminogen and fibrinogen are present in plasma at concentrations comparable to or much higher than vitronectin, no positive reactivity on Western blots for these proteins was observed (Figs. 4 and 5), indicating that vitronectin complexes or vitronectin alone may specifically bind to gC1qR.
Interaction Between gC1qR and Vitronectin Complexes in a Purified System—
Different vitronectin forms were tested for specific direct binding to gC1qR, immobilized to microtiter wells. Although plasma vitronectin exhibited hardly any interaction, the multimeric form as well as vitronectin-thrombin-antithrombin complex specifically bound to gC1qR in a saturable manner (KD ~20 nM) (Fig. 6A). Binding was completely inhibited in the presence of heparin but not chondroitin sulfate (data not shown), whereas the classical ligand C1q did not appreciably interfere with the binding of vitronectin to gC1qR (Fig. 6B). In the reverse situation, multimeric vitronectin did not interfere with binding of C1q to gC1qR (data not shown). This finding strongly argues in favor of different binding sites for C1q and multimeric vitronectin on the gC1qR molecule. To address this issue experimentally, the full-length recombinant

![Figure 4](http://www.jbc.org/)

**Fig. 4.** Plasma and serum proteins that bind to the recombinant gC1qR affinity column. A, fractions eluted from the affinity column were analyzed by SDS-polyacrylamide gel electrophoresis in 10% gels under reducing conditions; molecular weight markers (in thousands) in lanes 1 and 8 are indicated along the margins. The following materials were analyzed: unfractionated human serum, 0.063 μl (lane 2); TBS/EDTA wash buffer before elution of the serum-bound fraction (lane 3); bound fraction from serum, 3.1 μg (lane 4); bound fraction from plasma, 5.4 μg (lane 5); TBS/EDTA wash buffer before elution of the plasma-bound fraction (lane 6); and unfractionated human plasma, 0.063 μl (lane 7). The eluted proteins in lanes 4 and 6 were labeled a-i and analyzed by microsequencing (see Table I). B, the following fractions, which were retained on the gC1qR affinity column, were analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions followed by Western blotting using antibodies against plasminogen and vitronectin: serum-bound fraction (lanes 2, 7, and 12); unfractionated human serum (lanes 3, 8, and 13); plasma-bound fraction (lanes 4, 9, and 14); and unfractionated human plasma (lanes 5, 10, and 15). Molecular weight markers (in thousands) in lanes 1, 6, and 11 are indicated along the margins.

![Figure 5](http://www.jbc.org/)

**Fig. 5.** Analysis of serum and plasma fractions bound to gC1qR affinity column. The following samples were analyzed by SDS-polyacrylamide gel electrophoresis under reducing (A) and nonreducing (B) conditions, followed by Western blotting with antibodies against prothrombin, antithrombin, and fibrinogen as indicated: serum-bound fraction (lanes 2, 7, and 12); unfractionated human serum (lanes 3, 8, and 13); plasma-bound fraction (lanes 4, 9, and 14); and unfractionated human plasma (lanes 5, 10, and 15). Molecular weight markers (in thousands) in lanes 1, 6, and 11 are indicated along the margins.

**TABLE I**

| Band | Molecular mass (kDa) | Protein sequencing | Western blotting |
|------|---------------------|--------------------|-----------------|
| a    | 93.7                | Thrombin and antithrombin | Thrombin and antithrombin |
| b    | 74                  | ND*                | Vitronectin      |
| c    | 66.8                | ND*                | Vitronectin      |
| d    | 60.5                | Vitronectin        | Vitronectin      |
| e    | 58.2                | Antithrombin       | Antithrombin     |
| f    | 41                  | ND*                | Thrombin         |
| g    | 33.4                | ND*                | Thrombin         |
| h    | 74                  | ND*                | Vitronectin      |
| i    | 66.8                | ND*                | Vitronectin      |

*ND, not determined.
form of gC1qR and a mutant lacking 22 amino acids from the N terminus were modified by biotinylation and compared for binding to immobilized multimeric vitronectin and C1q. Although the intact gC1qR exhibited specific binding to vitronectin, the deletion mutant hardly interacted with the adhesion protein (Fig. 6C), and these characteristics were independent of the presence of C1q. These results suggest that the N-terminal region of gC1qR is involved in the interaction. Only a moderate difference in the binding of C1q to the truncated form of gC1qR compared to the wild-type was noted.

Finally, Raji cell membrane extract as well as gC1qR isolated thereof were tested for direct binding to immobilized multimeric vitronectin. In both cases appreciable specific binding of the receptor was noted, comparable in its extent to the binding of recombinant gC1qR to vitronectin performed in parallel (Fig. 7). These findings unequivocally define gC1qR as a novel binding protein for multimeric forms of vitronectin.

FIG. 6. Binding of multimeric vitronectin to gC1qR. A, microtiter wells were coated with recombinant gC1qR; various forms of vitronectin at the given concentrations were reacted with the wells, and the specific binding of plasma vitronectin (●), multimeric vitronectin (▲), and the ternary vitronectin-thrombin-antithrombin complex (▼) is shown (mean ± S.D., n = 4 of a typical experiment). B, specific binding of multimeric vitronectin to immobilized recombinant gC1qR in the absence (●) and presence of heparin (▲) or C1q (▼) was followed by ELISA. Note that vitronectin and C1q did not interfere with each other for interaction with gC1qR. C, C1q (▲) and multimeric vitronectin (▼) were immobilized to microtiter wells, and the specific binding of biotinylated recombinant gC1qR as well as the truncated mutant gC1qRΔN was determined in the absence (● and ▲) or the presence (▼) of exogenous C1q. The data represent the mean of duplicates of a typical experiment (<10% variation).

FIG. 7. Binding of Raji cell membrane-derived gC1qR to vitronectin. Multimeric vitronectin was immobilized onto microtiter wells, and binding of Raji cell membrane extract (ngC1q-R) and gC1qR isolated from this extract (mgC1q-R) was compared to binding of recombinant gC1qR (rgC1q-R) in parallel. The extent of binding was determined using two monoclonal antibodies (60.11 and 74.5.2) against gC1qR. Results were expressed as duplicates of a typical experiment.

DISCUSSION

Two cellular proteins have been shown to bind to the complement component C1q, and they are not only distinguishable by their respective molecular masses and structural characteristics but also by their ligand binding properties. The C1q receptor recognizes multiple ligands by low-affinity interactions with their collagen-type regions; these ligands include C1q, mannan-binding protein, and conglutinin or surfactant protein A (14). Because all these ligands can cross-compete with similar affinity for binding to the C1q receptor (15), it was renamed the "collectin" receptor and apparently serves important roles in intrinsic immunity through opsonization of pathogens and complement activation (16). Apart from this widely expressed cell surface protein, a second C1q-binding protein, designated gC1qR, has recently been described that is expressed on circulating blood cells (7), endothelial cells, and other tissues (8) and differs from the former one by high affinity recognition and its specificity for the globular heads of C1q (6). gC1qR is also found in soluble form in the circulation (at approximately 4 μg/ml), yet its functional role remains obscure. We were interested in searching for possible additional ligands of gC1qR, and the availability of recombinant forms allowed us to use an affinity approach. Surprisingly, we were able to unequivocally identify vitronectin and vitronectin-containing complexes as additional specific ligands, and this contention was proven by direct binding experiments in a purified system.

The recombinant forms of gC1qR expressed in Escherichia coli were functional because the expressed material (including gC1qRΔN) exhibited tetramerization and was indistinguishable from the Raji cell-derived gC1qR in high affinity binding to C1q as well as effective complement inhibition (6). In contrast to the C1q receptor, immobilized gC1qR predominantly bound vitronectin-containing material from serum, but hardly any serum C1q was retained on the affinity column, suggesting that gC1qR preferentially recognizes specific forms of the adhesion protein. Analysis of bound fractions revealed that particularly heparin binding forms of vitronectin bound to gC1qR, whereas plasma vitronectin was hardly retained. This indicates that acidic regions of gC1qR may exhibit affinity for complementary basic regions such as the heparin-binding site that is only exposed in "unfolded" forms of vitronectin (17). These forms include multimeric vitronectin (also derived from platelets; Ref. 10) or the ternary vitronectin-thrombin-antithrombin complex, the latter being the ultimate reaction product of thrombin in blood coagulation, which is significantly

6 B. Lim, B. Ghebrehiwet, and K. B. M. Reid, unpublished observations.
forms of vitronectin.

Heparan sulfate proteoglycans of the vessel wall can interact with multimeric forms of vitronectin (19), and luminal heparinoids of endothelial cells have been proposed to mediate binding and uptake of the ternary complex (11). In light of the present findings, gC1qR expressed on vascular cells seems to share certain properties with these proteoglycans and may very well be another candidate for specific binding and clearance of heparin binding forms of vitronectin.

In vitro experiments utilizing purified components substantiated critical features of the vitronectin-gC1qR interaction that support the aforementioned interpretation: only heparin-binding multimeric vitronectin or the isolated ternary complex bound to recombinant as well as Raji cell membrane-derived gC1qR in a specific manner, whereas plasma vitronectin hardly exhibited binding, reminiscent of the previous findings using the affinity approach. Moreover, heparin but not chondroitin sulfate totally blocked this interaction, indicating the involvement of the heparin-binding site of vitronectin. It was also found that C1q did not compete for binding of multimeric forms of vitronectin to gC1qR, indicating that different domains of the C1q-binding protein are able to recognize both ligands in an independent manner. This contention was corroborated by utilizing mutant forms of recombinant gC1qR, one of which lacked the N-terminal portion. This deletion mutant hardly interacted with vitronectin but was only moderately less effective compared with wild-type gC1qR in recognizing C1q. These findings strongly suggest that at least major parts of the binding site for vitronectin lie in an acidic stretch of the N-terminal region of gC1qR. Additional experimentation using domain-specific monoclonal antibodies or synthetic peptides is needed to unequivocally define these complementary binding sites. Due to the oligomeric nature of both components, it remains to be established whether their interaction is accomplished by multivalent species. As a possible consequence, multimeric vitronectin in soluble form or associated with extracellular matrix may thereby concentrate and induce clustering of gC1qR molecules, and the results in Fig. 7 as well as preliminary experiments with intact Raji cells suggest a possible role of gC1qR in adhesion to immobilized vitronectin. The issue of whether gC1qR is constitutively present as cell surface receptor is still controversial (20); however, cellular activation may result in increased surface expression of gC1qR when cells are allowed to become activated.4

Including gC1qR as a novel vitronectin-binding protein, at least four different types of cellular binding proteins for the adhesion factor have been described; integrins αvβ3 and αvβ5 expressed on endothelial and other cells have been implicated in cell adhesion and migration on vitronectin substrata (17, 21) or in the endocytosis of denatured vitronectin, respectively (22).

3 B. Ghebrehiwet, B. L. Lim, F. Festy, E. L. B. Peerschke, and K. B. M. Reid, submitted for publication.
4 K. B. M. Reid and B. Ghebrehiwet, unpublished observations.

Heparan sulfate proteoglycans on endothelial cells are able to mediate cell adhesion and spreading on immobilized vitronectin (18) and possibly mediate uptake, whereas recent data indicate that glycolipid-anchored urokinase receptor serves as an additional high affinity receptor for soluble multimeric forms of vitronectin (23, 24). Because heparin-competable binding of vitronectin to proteoglycans and αvβ5 has been described (11, 25), possible interference with the gC1qR interaction may be expected as well. Together, the functional properties of vitronectin as an indirect opsonin (26, 27), a participating component in apoptosis (28), and a major complement inhibitor are likely to be expressed in concert with gC1qR. The distribution pattern of vitronectin and its novel binding protein in the vascular system may very well indicate a functional linkage of gC1qR-bearing cells and vitronectin-rich extracellular matrix. Both components can thereby act in a cooperative fashion in processes of wound healing and immune defense.

Acknowledgments—The skillful technical assistance of C. Kost, F. K. Fung, and T. Schmidt is gratefully acknowledged.
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J. Biol. Chem. 1996, 271:26739-26744.
doi: 10.1074/jbc.271.43.26739

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