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A Recombinant Homotrimer, Composed of the α Helical Neck Region of Human Surfactant Protein D and C1q B Chain Globular Domain, Is an Inhibitor of the Classical Complement Pathway

Uday Kishore,* Peter Strong,† Michael V. Perdikoulis,‡ and Kenneth B. M. Reid2†

The first step in the activation of the classical complement pathway by immune complexes involves the binding of the six globular heads of C1q to the Fc regions of IgG or IgM. The globular heads of C1q (gC1q domain) are located C-terminal to the six triple-helical stalks present in the molecule, each head being composed of the C-terminal halves of one A, one B, and one C chain. The gC1q modules are also found in a variety of noncomplement proteins, such as type VIII and X collagen, precurcubin, hibernation protein, multimerin, Acrp-30, and saccular collagen. In several of these proteins, the chains containing these gC1q modules appear to form a homotrimeric structure. Here, we report expression of an in-frame fusion of a trimerring neck region of surfactant protein D with the globular head region of C1q B chain as a fusion to Escherichia coli maltose binding protein. Following cleavage by factor Xa and removal of the maltose binding protein, the neck and globular region, designated ghB3, formed a soluble, homotrimeric structure and could inhibit C1q-dependent hemolysis of IgG- and IgM-sensitized sheep erythrocytes. The finding that ghB3 is an inhibitor of C1q-mediated complement activation opens up the possibility of blocking activation at the first step of the classical complement pathway.

C1q plays a key role in the recognition of immune complexes, thereby initiating the classical complement pathway (CCP). The human C1q molecule (460 kDa) is composed of 18 polypeptide chains (6A, 6B, and 6C). The A chain (223 residues), B chain (226 residues), and C chain (217 residues) each have a short (3–9 residues) N-terminal region (containing a half cysteine residue involved in interchain disulfide bond formation), followed by a collagen-like sequence of ~81 residues and a C-terminal globular region (gC1q domain) of ~135 residues (1). Comparison of the mature chains shows that there are four conserved cysteine residues in each chain (at positions 4, 135, 154, and 171, as per the B chain numbering). The cysteine residues at position 4 in each of the chains are involved in the interchain disulfide bridging yielding the A-B and C-C subunits; the other three are considered to yield one intrachain disulfide bond and one free thiol group per C-terminal globular region. The interchain disulfide bonding yields six A-B dimer subunits and three C-C dimer subunits. The collagen-like sequences in the A and B chains of an A-B subunit form a triple-helical collagen-like structure with the equivalent sequence in one of the C chains present in a C-C subunit, to form a structural unit, of the composition ABC-CBA, which is therefore held together by both covalent and noncovalent bonds. Three of these structural units are then considered to associate, via strong noncovalent bonds in the fibril-like central portion, to yield the hexameric C1q molecule (2). The first component of complement C1 is a complex of three glycoproteins, C1q, C1r, and C1s. C1s and C1r interact to form a tetrameric proenzyme complex, C1r2-C1s2, which makes contacts with the C1q collagen region. Many activating ligands for C1, including immune complexes, bind to the gC1q domains; however, a number of nonimmune substances, such as DNA, C-reactive protein (CRP), serum amyloid protein (SAP), decorin, and some putative C1q receptors are thought to bind C1q via the collagen region. Binding of C1q to immune complexes (IgG or IgM) via the gC1q domain is considered to induce a conformational change in the collagen region of C1q, which leads to the autoactivation of C1r, which in turn activates C1s. The activated C1 complex then cleaves components C2 and C4 in the CCP. After C1 activation and removal of activated C1r2-C1s2 by C1 inhibitor, the collagen region appears to interact with cell surface receptors.

Human C1q shows only weak binding to the Fc regions of nonaggregated IgG (4 × 107 to 5 × 109 M⁻¹). Upon presentation of multiple, closely spaced Fc regions, as are found in immune complexes, the strength of binding of the hexameric C1q to IgG increases dramatically (1017 to 1021 M⁻¹) (3, 4). The precise binding
region of the IgG molecule for C1q is considered to be located in the C-terminal half of the C2 domain of IgG and, specifically, to three amino acids, Glu318, Lys320, and Lys322 which are highly conserved in different IgG isotypes (5). The charged residues Asp317, Glu418, and His420 in the C343 region of IgM have been proposed to form the binding site for the gC1q domain (6). Recent reports of recombinant production and characterization of the globular region of all three chains of C1q suggest that the gC1q domain is likely to be composed of three structurally and functionally independent modules, which retain multivalency in the form of a heterotrimer (7–9). The gC1q-like modules are also found in a variety of noncomplement proteins, which include the C-terminal globular regions of the human type VIII and type X collagen, precerebellin, the chipmunk hibernation proteins, multimerin, Acrp-30, and the sunfish inner-ear specific structural protein, called sacculus collagen (9, 10). In several of these proteins, the chains containing gC1q modules appear to form a homotrimetric structure. The crystal structure of the homotrimeric Acrp-30 (11) suggests that gC1q modules may assemble as C-terminal appendages to the collagen regions in the same way as the carbohydrate recognition domains (CRDs) present in the family of proteins called collectins. The members of the collectin family include SP-D and globular head region of C1q B chain, corresponding to the residues 90–226, which acts as a nucleation center for the trimerization of the CRDs (12). However, the gC1q domain, which has a very different fold than CRD, leads directly into the collagen region with no intervening neck region. The specific hydrophobic bonds within the sequence of the globular regions are considered to facilitate the interchain recognition and alignment of the three chains to yield a heterotrimetric (gabh, gbh, ghb) globular head structure, which, in turn, could act as a nucleation center for the trimerization of the triple-helical collagen region.

To further dissect the modular organization of the gC1q domain of human C1q, we addressed the question of whether a homotrimeric structure containing one type of globular region, as is seen in other members of the C1q family, can retain some biological functions. We made an upstream fusion of the trimerizing, α-helical, coiled-coil neck region of human lung SP-D with the C-terminal portion of human C1q, we addressed the question of whether a homotrimeric module as a homotrimeric structure, which, in turn, could act as a nucleation center for the trimerization of the C-terminal CRDs. The globular head region of the human C1q B chain (ghb) and the C-terminal collagen, precerebellin, the chipmunk hibernation proteins, multimerin, Acrp-30, and the sunfish inner-ear specific structural protein, called sacculus collagen (9, 10). In several of these proteins, the chains containing gC1q modules appear to form a homotrimetric structure. The crystal structure of the homotrimeric Acrp-30 (11) suggests that gC1q modules may assemble as C-terminal appendages to the collagen regions in the same way as the carbohydrate recognition domains (CRDs) present in the family of proteins called collectins. The members of the collectin family include SP-D and globular head region of C1q B chain, corresponding to the residues 90–226, which acts as a nucleation center for the trimerization of the CRDs (12). However, the gC1q domain, which has a very different fold than CRD, leads directly into the collagen region with no intervening neck region. The specific hydrophobic bonds within the sequence of the globular regions are considered to facilitate the interchain recognition and alignment of the three chains to yield a heterotrimetric (gabh, gbh, ghb) globular head structure, which, in turn, could act as a nucleation center for the trimerization of the triple-helical collagen region.

To further dissect the modular organization of the gC1q domain of human C1q, we addressed the question of whether a homotrimeric structure containing one type of globular region, as is seen in other members of the C1q family, can retain some biological functions. We made an upstream fusion of the trimerizing, α-helical, coiled-coil neck region of human lung SP-D with the C-terminal portion of human C1q. The globular head region of the human C1q B chain (ghb) and expressed in Escherichia coli linked to maltose-binding protein (MBP). The expressed recombinant polypeptide, composed of MBP, the factor Xa protease site, and the neck and ghb regions, was affinity purified using an amylose resin column and then cleaved with factor Xa to release a hybrid molecule (neck/ghb), designated gbh3. The gbh3 formed a soluble homotrimer in solution, preferentially bound aggregated IgG in ELISA, and inhibited gC1q-mediated complement activation. The finding that the recombinant gbh3 is an inhibitor of C1q-mediated complement activation opens up the possibility of blocking activation of the CCPs in a very early stage, and is consistent with the view that the globular region of C1q B chain is an independently folding module. The generation of a monomeric module as a homotrimeric structure also highlights the potential of the neck region of human SP-D as a trimerizing/multimerizing agent.

**Materials and Methods**

**Purification of human C1q**

Hemolytically active C1q was purified from human serum, using procedures previously described by Reid (13), with few modifications. Briefly, serum was diazylized against a 50-fold excess of distilled water overnight at 4°C. The resulting precipitate was harvested, solubilized in TE buffer (20 mM Tris-HCl and 5 mM EDTA, pH 7.4) containing 500 mM NaCl, and passed through a Q-Sepharose column (Pharmacia, Piscataway, NJ), which retained C1r/C1s and IgM. The C1q-enriched flowthrough was then applied to an SP-Sepharose column (Pharmacia), extensively washed with TE containing 150 mM NaCl to remove IgG. C1q was eluted using a 150- to 500-mM NaCl gradient, and the peak fractions were concentrated by ultrafiltration and further purified by Superox 6 gel filtration chromatography. The purity of C1q was assessed by SDS-PAGE (15% w/v) under reducing conditions where it appeared as three bands, corresponding to the A, B, and C chains of 34, 32, and 27 kDa, respectively. The final yield of purified C1q from 100 ml serum was ~2 mg.

**Construction of plasmid encoding the neck region of human SP-D and globular head region of C1q B chain**

The expression vector pMal-c2 (New England Biolabs, Beverly, MA), which contains the *E. coli malE* gene under the isopropyl β-D-thiogalactoside (IPTG)-inducible Ppromoter (coding for MBP), was used for expression. A Bluescript plasmid containing neck and CRD regions of human SP-D (12) was used to PCR-amplify the neck region as an *XbaI-MscI* fragment (170 bp). Using B chain cDNA as a template (1), the globular head region of the C1q B chain, corresponding to the residues 90–226, was PCR amplified as an *Smal-HindIII* fragment (FP, 5′-GGGGACTA CAAGGCCACCCCGAGAAA-3′, universal reverse primer, 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, 30 cycles). In a three-piece ligation reaction, pMal-c2 (*XbaI-HindIII* backbone), neck region (*XbaI-MscI*), and the globular region of C1q B chain (*Smal-HindIII*) yielded a new construct, designated pKBM-b, which comprised of the neck region/ghb linked to MBP and a factor Xa cleavage site.

**Expression and purification of the recombinant gbh3**

*E. coli* BL21 containing pKBM-b, was grown in Luria-Bertani medium with ampicillin (100 μg/ml) to an OD600 of 0.8 at 37°C and induced with 0.4 mM IPTG for 3 h. Cells were harvested by centrifugation, suspended in 50 ml lysis buffer (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 1% v/v Triton X-100, 1 mM EDTA, 1 mM PMSF, and 5% v/v glycerol) containing lysozyme (100 μg/ml) and PMSF (0.1 mM), and incubated on ice for 30 min. The cell lysate was then passed through a 60 Hz for 30 min, with an interval of 1 min (15 cycles) to disrupt the cells and shear the bacterial chromosomal DNA. After centrifugation at 16,000 × g for 30 min, the supernatant was collected and diluted 5-fold using column buffer I (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1% v/v Triton X-100, 1 mM EDTA, and 5% v/v glycerol) and loaded onto an amyllose resin column (50 ml bed volume; New England Biolabs) equilibrated with the column buffer. The column was washed successively with 3 bed volumes of column buffer I and 5 bed volumes of column buffer II (column buffer I without Triton X-100). The fusion protein was eluted with 100 ml of column buffer II containing 10 mM maltose. The peak fractions were pooled and diazylized against factor Xa buffer (20 mM Tris-HCl, 100 mM NaCl, 2 mM CaCl2, 5% v/v glycerol), and the concentration was adjusted to 1 mg/ml. Factor Xa (1 U/ml; New England Biolabs) was added (1 U factor Xa per 50 μg of fusion protein) and incubated overnight at 4°C. The factor Xa digest was loaded over a Q-Sepharose column and washed extensively with column buffer II (to remove unbound MBP, which elutes at 150 mM NaCl), and then gbh3 was eluted using a 0.1–1 M NaCl gradient. The peak fractions containing gbh3, eluted between 0.3 and 0.45 M. This pool was concentrated to 1 ml and loaded onto a Superose 12 gel filtration column (Pharmacia) equilibrated with 20 mM Tris-HCl, 100 mM NaCl, and 1 mM EDTA, pH 7.4. Fractions that eluted with an apparent molecular mass —60 kDa were pooled.

**Western blot analysis of the recombinant gbh3**

The gbh3 (~2 μg) was electrophoresed on a 15% (w/v) SDS-PAGE under reducing conditions and electrotransferred to polyvinyl difluoride transfer membrane. After blocking with 2% (w/v) BSA, the membrane was probed with rabbit anti-human C1q or anti-gb polycyclonal Abs (7:1:5000 dilution), followed by incubation with a goat anti-rabbit IgG-alkaline phosphatase conjugate (1:10,000 dilution). The blot was developed using the substrates, p-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate. The globular head region of native C1q (prepared after collagenase treatment) was used as positive control. BSA and human perdin were used as negative control proteins.

**N-terminal sequencing**

To confirm the N-terminal sequence of the recombinant protein, the purified gbh3 was applied to an Applied Biosystems (Foster City, CA) 470A
upon induction with 0.4 mM IPTG, the fusion protein, MBP-ghB3, accumulated as an overexpressed protein of ~60 kDa; lane 4, the MBP-ghB3, was affinity purified using amylose resin; lane 5, the MBP-ghB3 was then cleaved using site-specific factor Xa protease that yielded MBP (~40 kDa) and ghB3 (~20 kDa). B, Western blots of ghB3. Purified ghB3 was run on a SDS polyacrylamide gel (15% w/v) under reducing conditions, electrophoresed to polyvinyl difluoride membranes, and probed with specific Abs. Blot on left, lane 1, human C1q (prepared after collagenase digestion of intact human C1q) probed with a rabbit anti-human C1q polyclonal Ab (lane 1). Blot in the middle is ghB3 probed with rabbit anti-human C1q monoclonal Ab. Lane 2, BSA; lane 3, human properdin; lane 4, ghB3. Blot on right is ghB3 probed with rabbit anti-recombinant ghB Ab (7). Lane 5, BSA; lane 6, human properdin; lane 7, ghB3. C, SDS-PAGE (15% w/v) analysis of purified ghB3 under reducing and nonreducing conditions. The ghB3 was separated away from MBP using Q-Sepharose ion-exchange chromatography, which migrated at ~20 kDa under reducing (lane 1) as well as nonreducing conditions (lane 2).

**FIGURE 1.** A, SDS-PAGE (15% w/v) analysis of the expression and purification of the ghB3 that was expressed as a fusion to MBP. Lane 1, molecular mass markers; lane 2, lysate from cells before IPTG induction; lane 3, upon induction with 0.4 mM IPTG, the fusion protein, MBP-ghB3, accumulated as an overexpressed protein of ~60 kDa; lane 4, the MBP-ghB3, was affinity purified using amylose resin; lane 5, the MBP-ghB3 was then cleaved using site-specific factor Xa protease that yielded MBP (~40 kDa) and ghB3 (~20 kDa). B, Western blots of ghB3. Purified ghB3 was run on a SDS polyacrylamide gel (15% w/v) under reducing conditions, electrophoresed to polyvinyl difluoride membranes, and probed with specific Abs. Blot on left, lane 1, human C1q (prepared after collagenase digestion of intact human C1q) probed with a rabbit anti-human C1q polyclonal Ab (lane 1). Blot in the middle is ghB3 probed with rabbit anti-human C1q monoclonal Ab. Lane 2, BSA; lane 3, human properdin; lane 4, ghB3. Blot on right is ghB3 probed with rabbit anti-recombinant ghB Ab (7). Lane 5, BSA; lane 6, human properdin; lane 7, ghB3. C, SDS-PAGE (15% w/v) analysis of purified ghB3 under reducing and nonreducing conditions. The ghB3 was separated away from MBP using Q-Sepharose ion-exchange chromatography, which migrated at ~20 kDa under reducing (lane 1) as well as nonreducing conditions (lane 2).

**FIGURE 2.** SDS-PAGE (15% w/v) analysis of ghB3 under reducing conditions, following cross-linking in the presence of BS3. Lane 1, molecular mass markers; lane 2, ghB3 with no BS3 showing only monomer at ~20 kDa; lane 3, ghB3 with 0.01 mM BS3 showing some dimer at ~40 kDa; lane 4, ghB3 with 0.1 mM BS3 showing some dimer at ~40 kDa and trimer at ~60 kDa; lane 5, ghB3 with 1 mM BS3 showing mostly trimer at ~60 kDa.

**Results**

**Expression and purification of ghB3**

The globular head region of human C1q B chain, together with the neck region of human lung SP-D, was expressed as a soluble fusion to *E. coli* MBP. As shown in Fig. 1A, upon induction with 0.4 mM IPTG, the fusion protein, MBP-ghB3, accumulated as an overexpressed protein of ~60 kDa; lane 4, the MBP-ghB3, was affinity purified using amylose resin; lane 5, the MBP-ghB3 was then cleaved using site-specific factor Xa protease that yielded MBP (~40 kDa) and ghB3 (~20 kDa). B, Western blots of ghB3. Purified ghB3 was run on a SDS polyacrylamide gel (15% w/v) under reducing conditions, electrophoresed to polyvinyl difluoride membranes, and probed with specific Abs. Blot on left, lane 1, human C1q (prepared after collagenase digestion of intact human C1q) probed with a rabbit anti-human C1q polyclonal Ab (lane 1). Blot in the middle is ghB3 probed with rabbit anti-human C1q monoclonal Ab. Lane 2, BSA; lane 3, human properdin; lane 4, ghB3. Blot on right is ghB3 probed with rabbit anti-recombinant ghB Ab (7). Lane 5, BSA; lane 6, human properdin; lane 7, ghB3. C, SDS-PAGE (15% w/v) analysis of purified ghB3 under reducing and nonreducing conditions. The ghB3 was separated away from MBP using Q-Sepharose ion-exchange chromatography, which migrated at ~20 kDa under reducing (lane 1) as well as nonreducing conditions (lane 2).

**Binding specificity of ghB3 for heat-aggregated IgG and IgM**

C1q and ghB3 (0–1 μg/ml) in sodium carbonate buffer, pH 9.6, were coated to the wells of polysorb ELISA plates overnight at 4°C. After blocking with 2% (w/v) BSA for 2 h and subsequent washing, the plates were incubated with heat-aggregated IgG (10 μg/ml) or IgM (20 μg/ml) in TBS-NTP (50 mM Tris-HCl, 150 mM NaCl, 0.05% w/v Na2CO3, 0.05% v/v Tween 20, and 5 mM CaCl2) at 37°C. Following a 2-h incubation, the plates were washed, and anti-human IgG and IgM, which had been conjugated to alkaline phosphatase, were added (at a 1:10,000 dilution) to the appropriate wells. Following incubation for 2 h, the microtiter wells were developed using the substrate p-nitrophenyl phosphate, and A405 was measured. BSA was used as a control protein.

**Inhibition of C1q-dependent hemolysis by ghB3**

C1q hemolytic assays were essentially performed as previously described (7, 14). SRBC sensitized with either IgG (EAgG) or IgM (EAgM) were prepared in DGVB2 (isotonic Veronal-buffered saline containing 0.1 mM CaCl2, 0.5 mM MgCl2, 0.1% w/v gelatin, and 1% w/v glucose). The addition of human C1q (1 μg/ml) back to C1q-deficient serum (1:40 dilution in DGVB2) was sufficient to lyse >95% SRBC (EAgG or EAgM). Using a 1-μg/ml concentration of C1q, the experiments were performed to examine whether the binding of ghB3 to EAgG or EAgM resulted in inhibition of C1q-dependent hemolysis. Aliquots of EAgG or EAgM (107/100 μl) were coincubated with ghB3, MBP-ghB3, MBP, and rSP-D (0–10 μg) for 1 h at 37°C. The pretreated cells were then gently pelleted by centrifugation at 3000 × g for 2 min, washed, and resuspended in 100 μl of DGVB2. Each aliquot of SRBC was added to a mixture composed of 1 μg of C1q in 10 μl of DGVB2, 2.5 μl of C1q-deficient serum, and 87.5 μl of DGVB2. After a 1-h incubation at 37°C, the reaction was stopped by transferring the tubes to an ice bath and adding 0.6 ml ice-cold DGVB. The unlysed cells were pelleted by centrifugation, and A412 values of 100-μl aliquots of the supernatant were measured. Total hemolysis was assessed as the amount of hemoglobin released upon cell lysis with water (100%). The C1q-dependent hemolytic activity was expressed as a percentage of total hemolysis. Purified MBP-ghB3 (7) was used as positive control, whereas MBP and a recombinant fragment of human SP-D, composed of trimeric neck and CRD regions (rSP-D; Ref. 12), were used as negative control proteins.

**Chemical cross-linking and SDS-PAGE analysis**

The recombinant ghB3 (300 μg/ml concentration) was dialyzed overnight against 10 mM HEPES buffer, pH 7.5, containing 100 mM NaCl and 1 mM EDTA. An aliquot of dialysate (45 μl) was incubated with 5 μl of various concentrations of bis-(sulfosuccinimidyl) suberate (BS3; Perbio Science U.K., Chester, U.K.) for 30 min at room temperature. The cross-linking reactions were electrophoresed on a 15% (w/v) SDS-polyacrylamide gel under reducing conditions and stained with Coomassie blue R-250.
mM IPTG, the fusion protein accumulated as an overexpressed protein of \( \sim 60 \) kDa (lane 3). Following one-step affinity purification over an amylose resin column (lane 4), the neck/ghB fragment was cleaved away from MBP by using factor Xa (lane 5). MBP migrated at \( \sim 40 \) kDa and ghB3 at \( \sim 20 \) kDa, which is its monomeric size. After completion of factor Xa cleavage (as judged by SDS-PAGE analysis), the free ghB3 was further purified using Q-Sepharose column chromatography (Fig. 1C, lane 1). The final recovery of ghB3 from a start culture of 1 L bacterial cells was \( \sim 2–3 \) mg.

**Biochemical characterization of ghB3**

The ghB3 was recognized by rabbit anti-human C1q as well as anti-ghB polyclonal Abs (7), as judged by the Western blot (Fig. 1B). Automated N-terminal amino acid sequencing of the recombinant protein confirmed the presence of the neck region of human SP-D, preceded by eight Gly-Xaa-Yaa triplets, derived from the collagen-like region of human SP-D. When applied to a Superose 12 gel-filtration column, ghB3 eluted as an apparent trimer of \( \sim 60 \) kDa, immediately after BSA (data not shown). When examined by SDS-PAGE, the ghB3 ran as a monomer even under nonreducing conditions (Fig. 1C, lane 2), suggesting that the trimerization was not because of aberrant disulfide bridges between the ghB3 regions. The fact that the neck region was responsible for the homotrimerization was further confirmed by the chemical cross-linking experiment, where a spectrum of monomer (\( \sim 20 \) kDa), dimer (\( \sim 40 \) kDa), and trimer (\( \sim 60 \) kDa) bands could be seen (Fig. 2). Upon reaction with a cross-linking agent (BS3), the highest oligomeric species seen was a trimer when the reaction progressed to completion, whereas protein bands corresponding to monomeric, dimeric, and trimeric species were seen in partially cross-linked reactions. Higher oligomers were never observed. A range of BS3

**FIGURE 3.** Binding of heat-aggregated IgG and IgM to solid-phase-bound C1q and ghB3. Various concentrations of C1q and ghB3 were coated to ELISA plates and then incubated with a fixed concentration of heat-aggregated IgG (10 \( \mu \)g/well, A) or of IgM (20 \( \mu \)g/well, B) for 2 h at 37°C. The plates were washed, and the amount of each Ig binding to C1q and ghB3 was measured. All experiments were performed in duplicate, and BSA was used as a control protein. Both aggregated IgG and IgM were bound equally well by C1q, whereas ghB3 bound with more preferential affinity to the aggregated IgG than to IgM.
to inhibit C1q-dependent hemolysis. As shown in Fig. 4A, the addition of ~3.5 μg (58.3 pmol) ghB3 brought hemolysis of EA IgG down to <50%, whereas it required ~5 μg (41.7 pmol) of MBP-ghB to bring about similar effect. In case of EA IgM, it required ~7.5 μg (120 pmol) ghB3 to bring C1q-mediated hemolysis down to <50%, whereas it required ~10 μg (83.4 pmol) of MBP-ghB to compete with C1q to a similar extent. Normal serum (1:20 dilution) and C1q-deficient serum (1:40 dilution) were used as control for complete and background lysis, respectively. MBP did not interfere with C1q-dependent hemolysis. We also included rSP-D, containing trimeric neck and CRD regions, to rule out the possibility that the neck region of SP-D, on its own, could interfere with the hemolytic assay.

Discussion

The structural organization of C1q is characterized by a distinctive, trimeric globular domain (gC1q domain/module) situated at the C-terminal end of the triple-helical collagen region; these trimers multimerize to form a bouquet (9). Such gC1q modules are also found in a variety of noncomplement proteins such as human type VIII (15) and type X collagen (16), precerebellin (17), the chipmunk hibernation proteins (18), human endothelial cell protein, multimerin (19), a serum protein, Acrp-30 that is secreted from mouse adipocytes (20), the saccular collagen (21), and EMILIN (elastin microfibril interface-located protein), found in elastin-rich tissue such as blood vessels, skin, heart, and lungs (22). The chains containing these gC1q modules appear to form either a homotrimer (as in type X collagen, multimerin, Acrp-30, precerebellin, and saccular collagen) or a heterotrimeric structure (as in C1q and the hibernation protein, which both have three types of chain, and the type VIII collagen, which has two α1-chains and one α2-chain). In the structure of Acrp-30 (and TNF family), the N- and C-terminal ends are directly adjacent to one another, suggesting that gC1q domains might assemble as either N- or C-terminal appendages to the collagen region (10, 11). This modular organization of the gC1q domain in the C1q family of proteins is somewhat similar to that found in the collectin family (23). However, the collagen region in the collectins is joined to the globular, CRD region via a neck region (24). The neck peptide is composed of a coiled-coil of three α helices that accounts for the major part of the trimer interface. It is a 35-aa-long structural motif, responsible for the tight parallel association and trimerization of the three CRDs of SP-D (and other members of the collectin family), and also for the folding of the collagen chains into a staggered triple helix (25, 26). The recombinant CRDs, when expressed without an upstream neck region, fail to trimerize (12). In the C1q family of proteins, the gC1q domains lead directly into the Gly-Xaa-Yaa repeats of the collagen region, with no intervening neck region. The folding of collagen triple-helices is considered to nucleate at its C-terminal end, where a precise alignment of the three chains is established by the gC1q domain. The recombinant forms of two individual globular head regions (ghA and ghB), when coproduced with the \textit{E. coli} chaperone GroESL, have been reported to behave as monomers on gel filtration (8), unlike recombinant globular regions of collagen X (27, 28) and Acrp-30 (11), which have been shown to form stable homotrimmers. In a preliminary report of crystallization of the C1q globular head region, a fragment containing the three globular head regions (A, B, and C) was generated by digestion of native C1q with collagenase, further treated with neuraminidase to remove sialic acids, and alkylated with iodoacetamide to block free thiol groups. In the crystal diffracting to 1.8 Å, there is one heterotrimer per asymmetric unit (29). Thus, the heterotrimeric organization of the gC1q domain in native C1q suggests the presence of structural patches within the globular region of each chain that...
interact specifically during biosynthesis. This is further supported by the fact that the gC1q domains, prepared by collagenase digestion of intact C1q, are difficult to segregate into the individual ghA, ghB, and ghC fragments (30).

The three chains of human C1q show only 30% sequence identity on comparison with each other. When conservative replacement amino acids are also included, the conserved regions vary in length from a single to five or six residues. These conserved regions of hydrophobic amino acid residues are considered to be responsible for the general maintenance of the overall structure of the gC1q domain, rather than involved in binding to immune complexes (9). When the C-terminal sequences of human C1q A, B, and C chains (residue 90 onwards, based on the B chain numbering) are compared, ~27% of the residues are found to be completely conserved. These include three cysteine and several hydrophobic and neutral residues that form the scaffold of the gC1q domain and impart upon it a largely β sheet structure, as has been predicted from Fourier transform infrared spectroscopy and averaged structure predictions (31). The recently described crystal structure of recombinant, homotrimeric Acrp-30 has revealed an asymmetric trimer of β sandwich protomers, each of which has a ten-strand jelly-roll folding topology, which is also seen in the TNF family of proteins (11). This fold appears to be common to all the members of the C1q family of proteins. The trimer is bell shaped, with a wide base. The trimer contacts take place through a cluster of hydrophobic interactions near the base, whereas the trimer interface near the apex is largely hydrophilic. These features are in common with TNF family trimers (32). The Acrp-30 structure shows that the globular region forms stable trimers, stabilized by a central hydrophobic interface, suggesting a structural basis for the globular structure predictions (31). The recently described crystal structure of recombinant, homotrimeric Acrp-30 has revealed an asymmetric trimer of β sandwich protomers, each of which has a ten-strand jelly-roll folding topology, which is also seen in the TNF family of proteins (11). This fold appears to be common to all the members of the C1q family of proteins. The trimer is bell shaped, with a wide base. The trimer contacts take place through a cluster of hydrophobic interactions near the base, whereas the trimer interface near the apex is largely hydrophilic. These features are in common with TNF family trimers (32). The Acrp-30 structure shows that the globular region forms stable trimers, stabilized by a central hydrophobic interface, suggesting a structural basis for their role in triple-helical assembly of collagen regions. Four residues are conserved throughout the members of C1q and TNF families: Tyr161, Gln206, Phe327, and Leu342 (based on Acrp-30 numbering). Each residue seems important for the correct packing of the hydrophobic core of the protomer. Chemical modification studies have implicated two regions of the C1q globular domain in IgG binding (33); these are in the C1q B chain (site 1, localized to residues 114–129) and in the A and C chains (site 2, both around residue 160). Each of these maps to the exterior of the Acrp-30 trimer. These two C1q sites can also be mapped to two separate loops in the Acrp-30 crystal structure, although site 1 appears more attractive as a candidate binding surface. These observations (11, 33), together with previous studies (7, 8), strongly favored ghB as a candidate for homotrimerization (9). A few general conclusions can be drawn from the results described in this study: 1) a single globular head module of C1q does not appear to homotrimerize on its own, unlike other members of the C1q family; 2) engineering of a trimerizing, α helical coiled-coil, neck region of human SP-D, upstream to single-chain globular head, can yield a stable homotrimer; 3) the homotrimeric globular head of C1q B chain can block C1q-dependent hemolysis of erythrocytes; and 4) the physical and functional behavior of ghB implies that the B chain globular head is an independently folded module.

The expression and functional characterization of the ghB3 has also indicated that the C-terminal regions of C1q A, B, and C chains, which form the globular head region, are likely to have some independence of structure and function (7, 8) and there is a major contribution of the globular region of the C1q B chain in binding to Ig. The production of ghB3 also highlights the significance of the neck peptide in the trimerization of a heterologous module. This opens up the possibility of using the neck region (of human SP-D) to trimerize other low-affinity domains or modules, such as selectins, single-chain Abs, receptor molecules, etc., to generate high-affinity multimeric molecules. Although complete...


