Two Oligomeric Forms of Plasma Ficolin Have Differential Lectin Activity*  

Tomoo Ohashi and Harold P. Erickson†  
From the Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Ficolins are plasma proteins with binding activity for carbohydrates, elastin, and corticosteroids. The ficolin polypeptide has a collagen-like domain that presumably brings three subunits together in a triple helical rod, a C-terminal fibrinogen-like domain (fbg) similar to that of tenasin, which presumably has the binding activities, and a small N-terminal domain that we find to be the primary site for forming the ficolin oligomer. By sedimentation equilibrium we determined that the main plasma form, which we call big ficolin, had mass of 827,000 Da, consistent with 24 subunits. Little ficolin, about half this size, was obtained after binding to a GlcNAc affinity column. Electron microscopy of little ficolin showed a parachute-like structure, with a small globe at one end, corresponding to the 12 N-terminal domains, and the fbg domains clustered together at the ends of the collagen rods. Big ficolin was formed by the face to face fusion of the fbg domains of two little ficolins, leaving the rods and N-terminal domains projecting at opposite ends. Little ficolin maintained a high affinity for the GlcNAc column, and big ficolin had a low affinity or none. The binding sites for ligands may be obscured in this big ficolin oligomer, providing a regulation of their activity.

Ficolin was originally isolated as a protein from pig uterus membrane extracts that bound transforming growth factor-β (1). Two cDNA clones with very similar sequences, ficolin-α and -β, were obtained by screening a pig uterus cDNA library (2). More recently, ficolins have been identified from human blood as a corticosteroid binding protein, termed hucl (3), an elastin binding protein, termed EBP-37 (4), and a GlcNAc binding lectin, termed P35 (5). Ficolin cDNAs, termed human ficolin (6), ficolin-1 (7), and P35-related gene (8), were also cloned. cDNA sequences (some of them are partial) suggest that there are two human ficolin genes. One gene codes for human ficolin, ficolin-1, and P35-related gene (which are different names given to this gene product), and the other codes for P35, EBP-37, and hucl. It is not yet clear how these relate to the two pig genes for α and β ficolins.

The amino acid sequence showed that ficolins consist of a short N-terminal domain, a middle collagen-like domain, and a C-terminal fibrinogen-like (fbg) domain (2, 5–7). A similar three-domain structure applies to complement protein C1q and the collectins, which include mannose binding protein, conglutinin, and pulmonary surfactant proteins A and D (SP-A and SP-D) (9–12). These proteins differ from ficolin in that their C-terminal domain is a C-type lectin instead of fbg, but they all have a middle collagen domain and a small N-terminal domain. The collagen domains assemble these proteins into trimers, and electron microscopy shows that four or six trimers are connected together by the N-terminal domain, leaving the C-terminal lectin domains to project in a multimeric array (13–17).

Complement proteins like C1q and collectins play roles in immune defense. Collectins bind to carbohydrates on the surface of bacteria and viruses and activate phagocytosis (10, 11). It has been indicated that collectin binding to carbohydrate ligands enhances opsonic activity via the C1q-collectin receptor complex (18, 19). Remarkably, ficolins have been implicated in a similar role, in that human plasma ficolin (P35) is a lectin that binds to the carbohydrate of bacterial surface (20) and enhances opsonic activity of polymorphonuclear neutrophils (5).

The binding activities of the collectins are in the C-terminal lectin domain, and it is reasonable to assume that the binding activity ficolin is in the C-terminal fbg domain. Fibrinogen-like domains are found in a number of proteins including fibrinogens β and γ and the tenasins. The ficolin fbg domain is most similar to those of the Drosophila signaling protein Scabrous and the tenasins. See Yee et al. (21) for the crystal structure of the fbg domain from fibrinogen-γ.2

An early study of pig ficolin demonstrated that ficolin secreted by transfected cells was assembled into disulfide-bonded oligomers, but these were not characterized, and there has been little study of the structure of natural ficolins. In the present study, we have purified ficolin from pig plasma using antibody and N-acetyl-D-glucosamine (GlcNAc) affinity columns. We have determined its molecular weight and hydrodynamic parameters by gel filtration chromatography, glycerol gradient sedimentation, and sedimentation equilibrium and demonstrated a unique structure by electron microscopy.

EXPERIMENTAL PROCEDURES

Bacterial Expression Proteins—The fbg domains of pig ficolin-α ((m)CLTGP...FRAT*; (m) indicates an initiation methionine not part of the ficolin sequence and * indicates the natural stop codon) and ficolin-β ((m)CATGP...VRLT*) were cloned into pET11 expression vectors as described for the tenasin fbg domain (22). Pig ficolin-α and -β cDNAs, kindly provided by Dr. Hidenori Ichijo, The Cancer Institute, Japan, were used as templates for polymerase chain reaction. Following expression in Escherichia coli, the ficolin fbg domains were entirely in the insoluble pellet of the bacterial lysate, as was TNfbg (22). The pellet was washed twice with lysate buffer and then resuspended in 4 M guanidine HCl in 20 mM Tris buffer, pH 8.0, containing 5 mM dithio-

---

* This work was supported by National Institutes of Health Grants AR42180 and CA47056. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 919-684-6385; Fax: 919-684-3687; E-mail: H.Erickson@cellbio.duke.edu.

1 The abbreviations used are: fbg, fibrinogen-like domain; SP-A and SP-D, surfactant protein A and D, respectively; TBS, Tris-buffered saline; PAGE, polyacrylamide gel electrophoresis.

2 For an updated version on-line of the structure-based sequence alignment and phylogenetic tree of fbg domains from different proteins, see http://www.bmsc.washington.edu/people/teller/suppl.html.
threitol. The sample was dialyzed against 6 M urea in 20 mM Tris buffer, pH 8.0, containing 1 mM dithiothreitol. The protein was renatured by dialyzing slowly against 20 mM Tris buffer, pH 8.0, containing 1 mM reduced and 0.3 mM oxidized glutathione. About 40% of the ficolin βfg protein was recovered in the soluble supernatant and was further purified by chromatography on a Mono Q column (Pharmacia Biotech Inc.), where it eluted at 0.24 M NaCl. However, gel filtration indicated that the final product was heterogeneous oligomers, and it did not bind the GlcNAc column, so it may not be properly folded. It was nevertheless suitable for making an antibody.

For preparation of polyclonal antibody Fio4325, a rabbit was immunized with 500 μg of ficolin in complete Freund's agent, followed by four boosts of 250 μg in incomplete Freund's. Antibodies were collected after the third and the fourth boosts. A similar antibody, Fio4326, was raised against pig ficolin-β fg domain.

**Purification of Ficolin Using Antibody Affinity Chromatography**—IgG was purified from the ficolin-α antiseraum by ammonium sulfate precipitation and DEAE-Sepharose chromatography (23). About 20 mg of purified IgG was then cross-linked to 15 ml of Affi-Gel HZ (Bio-Rad). Pig plasma (100 ml, collected in 1% citrate) was brought to 30% saturated ammonium sulfate, left at room temperature for 1 hr, and centrifuged at 11,100 × g, for 15 min. The precipitate was resuspended in 50 ml of 20 mM Tris buffer, pH 8.0, and passed over the antibody affinity column. The column was washed with 75 ml of 20 mM Tris buffer containing 0.15 M NaCl (TBS), and eluted with 2 bed volumes of 0.5 M glycine, pH 2.8. The eluate was neutralized immediately with 1 M Tris and precipitated again with ammonium sulfate to concentrate. The pellet was resuspended in 0.5 ml of 20 mM Tris buffer, pH 8.0.

**Purification of Ficolin Using a GlcNAc Affinity Chromatography**—100 ml of citrated pig plasma was run over a 10-ml GlcNAc-agarose column (Sigma) that had been washed with TBS. The column was washed with 5 bed volumes of TBS and eluted with 2 bed volumes of TBS containing 0.15 M GlcNAc, and then 2 bed volumes of TBS containing 0.8 M GlcNAc. Fractions from the GlcNAc column were contaminated with anti-carbohydrate IgG and IgM, which were removed by a two-step final purification on a resource Q column (Pharmacia). The ficolin eluted from the GlcNAc column was dialyzed into Tris buffer containing 0.2 M NaCl and passed through a 1-ml resource Q column. Most ficolin and IgG were in the flow-through, whereas IgM bound to the column. The flow-through was diluted with 20 ml Tris buffer to give a final NaCl concentration of 0.1 M and then passed through the column again to purify the ficolin. IgG was mostly in the flow-through, and ficolin eluted at 0.2–0.24 M NaCl, well separated from the IgG.

SDS-PAGE was performed using standard techniques. For Western blots proteins were transferred to Immobilon (Millipore, Bedford, MA) using a semi-dry electrophoret (Multiphor II, Pharmacia-LKB). Blots were stained with the polyclonal antiserum against pig ficolin-α, and developed with horseradish peroxidase secondary antibody.

**Hydrodynamic Parameters and Molecular Weight**—The Stokes radius (R_s) of ficolin was estimated by gel filtration on a Superose 6 column (Pharmacia, 1 × 31 cm) at a flow rate of 0.5 ml/min. The elution buffer was TBS, and the column was calibrated with standard proteins of known R_s (thyroglobulin, 8.5 nm; aldolase, 4.81 nm; bovine serum albumin, 3.55 nm).

The sedimentation coefficient was estimated by glycerol gradient sedimentation. The samples were sedimented on 15–40% glycerol gradients in 0.2 M ammonium bicarbonate at 32,000 rpm for 16 h in a Beckman SW-50.1 rotor. The glycerol gradients were calibrated with standard proteins of known S value (thyroglobulin, 19 S; catalase, 11.3 S; bovine serum albumin, 4.6 S).

The molecular weight was estimated from combined gel filtration and gradient sedimentation using Equation 1 (24).

\[
M_s = 4N_s/6 + (N_s - 1)(1 - v_s) = 4.204 \times 10^5 \text{ S} \times R_s
\]

where \(M_s\) is molecular weight, \(N_s\) is Avogadro's number, \(\eta_p\) is viscosity of the solvent (0.01 poise), \(v_s\) is partial specific volume of the protein (assumed to be 0.73 cm^3/g), \(\rho\) is density of solvent (1.0). For the numerical formulation on the right \(S\) is in Svedberg units and \(R_s\) is in nm.

Sedimentation equilibrium analysis was performed with a Beckman XL-A analytic ultracentrifuge. Purified big and little ficolins were dialyzed against 20 mM Tris buffer, pH 8.0, containing 0.1 M NaCl, and centrifuged at 20 °C at 3,000 and 5,500 rpm. The protein gradient along the cell was monitored as absorbance at 235 or 230 nm. Typical times to reach equilibrium were 24 h for big ficolin and 18 h for little ficolin.

A base line, corresponding to a few percent of total absorbance, was determined after centrifuging the samples at high speed and was subtracted from the measurements. The corrected absorbance profiles were fit to a single molecular species using software provided with the XL-A centrifuge.

**Electron Microscopy**—For rotary shadowing, samples from the glycerol gradient fractions were sprayed onto freshly cleaved mica, dried in vacuum, and rotary shadowed with platinum (25). For negative staining, a drop of protein was applied to a glow discharged carbon-coated grid, and 3 drops of 2% uranyl acetate were flushed over the grids. The last drop was dried with filter paper. The length and diameter measurements of stalk and globular domains were made on micrographs of negatively stained samples printed at 300,000 × magnification.

**RESULTS**

**Purification of Plasma Ficolin by Antibody or GlcNAc Affinity Chromatography**—The polyclonal antibody against pig ficolin-α βfg reacted strongly against the bacterial expressed pig ficolin-α βfg domain, and more weakly against the ficolin-β expression protein (the weak higher molecular weight bands in lanes 5 and 6 are contaminating bacterial proteins reacting with our polyclonal antibody) (Fig. 1). This antibody also labeled a broad 35-kDa band in pig plasma. The ficolin in crude pig plasma migrated at a higher molecular weight than did ficolin from the ammonium sulfate cuts or the more purified fractions. The slower migration may be due to the large amount of protein loaded in these fractions. The bands in Fig. 1 appear more diffuse than in other preparations (but note that the higher concentration starting material in Figs. 4 and 5 are also diffuse). This probably reflects the larger amount of protein loaded here and an excess staining by the antibody. However, the ficolin bands are always rather diffuse in our preparations, and this has been noted in previous reports (2, 7). The diffuse- ness of the ficolin bands may be partially due to glycosylation, since treatment with glycosidase was reported to sharpen the bands (2, 7). Pig plasma ficolin was precipitated by 30% saturated ammonium sulfate (Fig. 1) and then purified on the ficolin-α antibody affinity column. Analysis by gel filtration and sedimentation indicated that this antibody purified ficolin was almost exclusively the big ficolin oligomer (see below). The antibody purified ficolin contained several contaminating bands when analyzed by SDS-PAGE (not shown).

Following the recent report that a human plasma ficolin binds to GlcNAc affinity columns (5), we tested pig plasma ficolin for similar lectin activity. As shown in Fig. 2, pig plasma ficolin also bound to the GlcNAc column. We discovered that some of the ficolin eluted with 0.15 M GlcNAc, and a second fraction eluted at 0.8 M GlcNAc. The ficolin eluted from the column with GlcNAc was contaminated with proteins corresponding to immunoglobulin heavy and light chains, probably antibodies against carbohydrate epitopes. These contaminants were removed by chromatography on resource Q (Fig. 2B).
Western blot analysis indicated a variable fraction of the ficolin in plasma did not bind the GlcNAc column and was recovered in the flow-through. With two lots of pig plasma there was no binding of ficolin to the column, whereas with others 80–90% plasma ficolin bound to the column. ~70% bound ficolin eluted at 0.15 M GlcNAc, and this was identified as big ficolin, as described below. The remainder eluted at 0.8 M GlcNAc and was identified as little ficolin.

Size and Hydrodynamic Properties of the Native Ficolin Oligomers—When run on non-reducing SDS-PAGE both big and little ficolins showed the same molecular mass of approximately 400 kDa (Fig. 3). Antibody affinity purified ficolin also had the same molecular mass. On 12% acrylamide gels, non-reducing SDS-PAGE revealed a small fraction (10–20%) of monomeric 35-kDa ficolin, in addition to the 400-kDa band. We conclude that most of the ficolin is covalently cross-linked by disulfide bonds into 400-kDa oligomers (which correspond to a molecular mass of ~400 kDa). Antibody affinity purified ficolin also behaved identically on the Resource Q purification.

Gel filtration chromatography on Superose 6 was used to analyze ficolin in whole plasma, as well as purified big and little ficolins (Fig. 4). The ficolin peak from plasma eluted slightly ahead of thyroglobulin, at $R_S = 10.5$ nm (Fig. 4A). The GlcNAc affinity purified big ficolin eluted at the same $R_S$ (Fig. 4B) as did the antibody purified ficolin (not shown). In contrast, purified little ficolin eluted later, at $R_S = 7.3$ nm (Fig. 4C).

The same samples were analyzed by sedimentation on glycerol gradients (Fig. 5). Purified big ficolin sedimented as a sharp peak at 19.5 S and purified little ficolin sedimented at 12 S (Fig. 5, B and C). The ratio of these sedimentation coefficients to those of unhydrated spherical proteins of the same mass was 1.7 ($f_{\text{g}}/f_{\text{min}} = 1.7$), which is characteristic of moderately elongated proteins (26). In contrast, the ficolin in whole plasma sedimented as a very broad peak. There was substantial ficolin at the 19.5 S position of purified big ficolin and none at the position of little ficolin, but the majority of ficolin in plasma sedimented faster in a broad peak. This sedimentation implies that the ficolin in plasma is in the form of big ficolin oligomers and larger aggregates. These larger aggregates were not obvious on the gel filtration, perhaps because big ficolin itself elutes close to the void volume.

The $R_S$ estimated from gel filtration and the $S$ from gradient sedimentation were used to estimate the mass of native ficolins using the Svedberg equation (see “Experimental Procedures”). Big ficolin (860,000 Da) was approximately twice the size of
little ficolin (368,000 Da) (Table I).

A more rigorous estimate of the mass was obtained by sedimentation equilibrium of purified big and little ficolin. Computer-derived curves based on the assumption of a homogeneous single species gave excellent fits for both samples (Fig. 6). Big ficolin fit a mass of 827,000 Da and little ficolin 387,000 Da (Table I). For technical reasons the value obtained for big ficolin is considered more reliable. We conclude that big ficolin is a dimer of little ficolins.

Electron microscopy of rotary shadowed ficolin revealed several structural variations of both big and little ficolins (Fig. 7). Big ficolin typically appears as a large central cluster, rectangular in profile, with two small globes projecting at opposite sides (Fig. 7, A and B). More disrupted structures are occasionally seen (Fig. 7, C–G), as well as some larger aggregates (Fig. 7H). Little ficolin frequently appeared as a parachute-like structure that closely resembled half a big ficolin as follows: a large cluster at one end corresponding to half the central cluster of large ficolin, with a single small globe extended from it (Fig. 7, I–M). The most revealing images are those in which the large cluster appears to be disrupted, with one to four globular domains separated out (Fig. 7, J–M). These are connected to the small globe by thin rods that are only sometimes visible. We conclude that the basic structure of little ficolin comprises four globular domains on the end of long thin rods, with all four rods connected into the small globe at the other end (Fig. 7F). Interpretive drawings of the images are given in Fig. 10.

Electron micrographs of negatively stained big ficolin (Fig. 8) confirmed the structures seen with rotary shadowing (Fig. 7, A and B) and provide higher resolution images of the globular domains and rods. The central cluster appeared more compact in the negatively stained images, suggesting partial disruption when molecules are dried on mica. The small globes are quite visible, as are some of the thin rods linking them to the central cluster. We have generally found negative stain to give the most reliable high resolution structural information, and our measurements are mostly taken from these. The most accurate number, because it is the largest, is the total length from the outside of the two small globes, which is 40 nm. Each small globe is about 3 nm in diameter, and the central cluster measures 10 nm along the axis connecting the small globes, and is 13 nm wide. This leaves 12 nm for the rods measured from the surface of the small globe to the central cluster. We were not able to identify partially disrupted ficolins in negative stain, but rotary shadowed images showing globular domains separated from the central cluster indicated a diameter of 9 nm, after subtracting 2 nm for the shell of metal.

**Table I**

|                  | $R_g$ | $S$ | $M_r$ (S–M) | $M_r$ (sed eq) |
|------------------|-------|-----|-------------|----------------|
| Big ficolin       | 10.5  | 19.5| 861,000     | 827,000        |
| Little ficolin    | 7.3   | 12  | 368,000     | 387,000        |

**Fig. 5.** Glycerol gradient sedimentation of pig plasma ficolin. Samples were sedimented on 15–40% glycerol gradients at 32,000 rpm for 16 h in a Beckman SW-50.1 rotor. A, whole pig plasma stained with antiserum. B, purified big ficolin stained with Coomassie Blue. C, purified little ficolin stained with Coomassie Blue. The big and little ficolin samples used here had been purified by resource Q chromatography after elution from the GlcNAc column. *Lane numbers* correspond to gradient fraction numbers. *S*, starting material. Fraction 7 is about 19 S (thyroglobulin); fraction 9 is about 11.3 S (catalase); fraction 12 is about 4.6 S (bovine serum albumin).

**Fig. 6.** Sedimentation equilibrium of big and little ficolins. A, big ficolin and B, little ficolin were sedimented at 3,000 and 5,500 rpm, respectively, at 20 °C. Initial concentrations were 0.1 and 0.05 mg/ml for big and little ficolin. The circles are data points measured at 235 or 230 nm after equilibrium was established. The lines are the best fit for a single molecular species, giving a mass of 827,000 Da for big ficolin 387,000 Da for little ficolin.
and could be eluted again by 0.8 M GlcNAc. Matsushita et al. (5) reported that the lectin activity of ficolin required calcium, but our initial binding results were obtained with citrated plasma, which should have chelated the calcium. To test this further we dialyzed purified little ficolin into buffer containing 5 mM calcium or 10 mM EDTA. The ficolin bound to the GlcNAc column in both buffers, suggesting that calcium was not necessary for the lectin activity.

We made several attempts to convert little ficolin into large and visa versa. The addition of 0.8 M GlcNAc to purified big ficolin or to plasma did not change the gel filtration pattern of ficolin. Little ficolin was not detected in native plasma, but only after passing it over the GlcNAc column, so it appears to have been generated on the column. Once generated, however, it appears to be quite stable, with no tendency to re-assemble into big ficolin. The sedimentation equilibrium analyses were done 2 weeks after the purification, demonstrating the long term stability of the two forms.

**DISCUSSION**

Our model of ficolin is shown schematically in Figs. 9 and 10. The basic structural unit is a trimer, comprising a thin collagen-like rod with globular domains projecting at each end. Four trimeric units are joined at the small N-terminal domains to form a little ficolin oligomer. The larger C-terminal fbg domains cluster at the other end of the collagen rod. Two little ficolins join face to face at these fbg domains to form a big ficolin, which has 24 subunits.

The model agrees well with the 827,000 Da mass we measured for big ficolin. With 24 subunits the mass per subunit should be 34,458. Mature pig ficolin-α consists of 297 amino acids making a polypeptide chain of 32,044 Da ((2) but N-terminal amino acid microsequencing showed the mature peptide to begin at LDT (7)). There are three N-linked glycosylation sites in pig ficolin-α, one of which is conserved in all ficolins. The 34,458 Da estimated for the native subunit is 2,400 Da larger than the mass of the polypeptide, which is about the mass that would be added by a single carbohydrate cluster.

The amino acid sequence of ficolin indicates three independently folding domains (2, 5–7). The middle domain is most important for the structure. The 54 amino acid segment (GCPG... KGES) comprises 28 collagen-like GXY repeats,
The Structure of Ficolin

**Fig. 9. Schematic diagram of the structural organization of ficolin.** A, the trimeric ficolin unit consists of a short N-terminal domain, the middle collagenous domain, which forms an extended triple helical rod, and the three C-terminal fbg globular domains. The subunits of this trimer may be linked by disulfides near the fbg domain. B, little ficolin is a tetramer of trimeric subunits assembled at the N-terminal domains, involving additional disulfides. The fbg domains must be densely packed within the central cluster to fit the ~13-nm width seen in negative stain. The diagram on the right illustrates a possibility for close packing of ~4-nm globular fbg domains. In this diagram there is a 2-fold axis of symmetry on the long axis of the molecule. C, big ficolin is formed by the face to face fusion of the fbg clusters of two little ficolins.

which presumably form a three-stranded collagen helix. This collagen rod should have a length of 15.4 nm, based on the 0.286 nm/merid acid distance in the collagen triple helix (17). The thin collagen rods are scarcely visible in the electron micrographs, but it is clear that the 15.4 nm collagen rod is much longer than the 12 nm distance between the small and large globular domains. We conclude that the globular domains fold back over the collagen rod and that the termini of this rod are buried within them (Fig. 9).

On the N-terminal side of the collagen domain are the first 25 amino acids (APAL...SILR), which probably contribute to a small globular domain. Our electron micrographs show the four collagen rods connected at the small 3-nm globe (see Fig. 10 for schematic drawings). We conclude that the N-terminal segments of the 12 subunits are joined in this domain. Since ficolin, SP-D and conglutinin all have a small N-terminal domain that makes tetramers of collagen-based trimers, we looked for similarities. The N-terminal sequences of SP-D and conglutinin were 65% identical to each other, but only 20 and 25% are identical to ficolin. Even this limited sequence identity suggests that these proteins may share a common structural motif for bonding the 12 subunits, but this structure is not known for any of them.

**Fig. 10. Schematic diagrams of big and little ficolins matching electron micrographs.** Each trimeric unit is represented by a lollipop structure, whose rod is the collagen triple helix and whose large globular domain contains the three fbg domains. Four of these are bonded at the N-terminal domain to make little ficolin (A–E), and two little ficolins are joined at the fbg domains to make a big ficolin (F–I). Molecules are shown in various states of disruption to model the images seen by electron microscopy.

The C-terminal 221 amino acids (WETE...FRAT) form the fbg domain (the first few amino acids do not align with fbg domains of other proteins, and may be a spacer). The fbg domain from γ-fibrinogen has recently been resolved at atomic resolution by x-ray diffraction, and it measures 3.8 × 4.4 × 5.6 nm (21). Each collagen rod will carry three fbg domains at its end, which together should measure about 5.6 × 8 nm (Fig. 9A). This would correspond to the ~9-nm globular domains seen in disrupted molecules (the rotary shadowing may exaggerate the diameter). The ~10-nm thickness of the central cluster could accommodate the face to face junction of two domains along the long axis (Fig. 9C). It is more of a challenge to fit four of these trimeric units, each about 8 nm across, into the 13-nm width of the central cluster. However, if the trimeric units are rotated to achieve maximum packing of the 12 fbg domains, they will just about fit into a 13-nm wide cluster. Our speculative model for this packing is shown in Fig. 9B.

The central cluster of big ficolin appears to involve the face to face fusion of the fbg domains from two little ficolins. This fusion is apparently non-covalent, since non-reducing SDS-PAGE gives the same 400-kDa mass for both little and big ficolin. What is the disulfide bonding arrangement? There are two cysteines in the N-terminal domain (one is actually the second residue of the collagen rod, which we conclude is buried in the globular domain) available for disulfide cross-linking. It is difficult to imagine that these two cysteines could form a network of disulfides linking all 12 subunits. There are two additional cysteines in the C-terminal domain that could be involved in inter-chain disulfides, one just before and one within the fbg domain (we ignore the four cysteines making internal disulfides within fbg (21)). A useful analogy may be seen in SP-D, which also has two cysteines in the N-terminal domain but has no others available for inter-chain disulfides. On non-reducing SDS-PAGE SP-D shows trimers, probably two subunits from one collagen-linked trimer and one from another (17). We speculate that the two cysteines in the N-terminal domain are sufficient to form trimers, as in SP-D, but the C-terminal cysteines of ficolin may be necessary to link all 12 subunits into a single unit.
It seems likely that the lectin activity and other binding functions are in the fbg domain, so the structure of big ficolin raises a question. Why would these binding domains be buried in the center of the molecule? We suggest this may be a control mechanism. Concealed binding activity is actually consistent with our observation that big ficolin binds only weakly to the GlcNAc column and loses its affinity completely after elution and dialysis. In contrast, we observed that little ficolin bound tightly to the GlcNAc column, which is consistent with the full exposure of its 12 fbg domains, permitting multiple attachments of a single molecule. It is possible that the initial binding of big ficolin was due to partially disrupted molecules with only one or two fbg domains exposed (Fig. 10, G, H, and I) and that after purification these re-associate to the fully closed molecule (Fig. 10F). The transition to fully active little ficolin may occur in steps.

We only detected little ficolin after interaction with the GlcNAc column, so it is possible that contact with the column caused the formation of the active little ficolin. We can only speculate on the source of the little ficolin. Native ficolin in plasma is mostly big ficolin and larger aggregates. A certain fraction of the big ficolins may be partially disrupted, extending one or two arms to make a partially active form. This would probably be reversible, the molecule returning to the fully closed form in the absence of outside forces. But if one of these extended arms did bind a ligand, this could trap that arm and prevent re-folding. The trapped ficolin could then become further disrupted, leading to additional trapped arms and eventually a fully active little ficolin. This could be particularly attractive as a mechanism for binding a multivalent ligand like a bacterial surface.

Ficolin was originally identified as a transforming growth factor β binding protein (1), but this binding activity has not been confirmed for recombinant ficolin or for plasma ficolin. Plasma ficolin has been reported to bind elastin and gelatin (7) and corticosteroids (3). We would predict that these binding activities are in the fbg domains and that they will be differentially activated in big and little ficolin.

Acknowledgments—We thank Dr. Harvey Sage, Biochemistry, Duke University Medical Center, for performing the analytical ultracentrifugation and the computer analysis of the results. We thank Dr. Virginia A. Lightner, Medicine, Duke University Medical Center, for helpful discussions and advice.

REFERENCES
1. Ichijo, H., Ronnstrand, L., Miyagawa, K., Ohashi, H., Heldin, C.-H., and Miyazono, K. (1991) J. Biol. Chem. 266, 22459–22464
2. Ichijo, H., Hellman, U., Wernstedt, C., Gómez, L. J., Claesson-Welsh, L., Heldin, C.-H., and Miyazono, K. (1993) J. Biol. Chem. 268, 14555–14513
3. Edgar, P. F. (1995) FEMS Lett. 375, 159–161
4. Harumiya, S., Omoshi, A., Sugita, T., Fukumoto, Y., Tachikawa, H., and Fujimoto, D. (1995) J. Biochem. (Tokyo) 117, 1029–1035
5. Matsushita, M., Endo, Y., Taira, S., Sato, Y., Fujita, T., Ichikawa, N., Nakata, M., and Mizuochi, T. (1996) J. Biol. Chem. 271, 2448–2454
6. Lu, J., Tay, P. N., Kon, O. L., and Reid, K. B. (1996) Biochem. J. 313, 473–478
7. Harumiya, S., Takeda, K., Sugita, T., Fukumoto, Y., Tachikawa, H., Miyazono, K., Fujimoto, D., and Ichijo, H. (1996) J. Biochem. (Tokyo) 120, 745–751
8. Endo, Y., Sato, Y., Matsushita, M., and Fujita, T. (1996) Genomics 36, 515–521
9. Thiel, S., and Reid, K. B. M. (1989) FEMS Lett. 250, 78–84
10. Holmskov, U., Malhotra, R., Sim, R. B., and Jensenius, J. C. (1994) Immunol. Today 15, 67–74
11. Hoppe, H.-J., and Reid, K. B. M. (1994) Protein Sci. 3, 1143–1158
12. Kuroki, Y., and Voelcker, D. R. (1994) J. Biol. Chem. 269, 25943–25946
13. Knobel, H. R., Villiger, W., and Isliker, H. (1975) Eur. J. Immunol. 5, 78–82
14. Andersen, O., Friis, P., Nielsen, E. H., Vilsgaard, K., Leslie, R. G. Q., and Svehag, S.-E. (1992) Scand. J. Immunol. 36, 131–141
15. Voss, T., Eistetter, H., and Schaffer, K. P. (1988) J. Mol. Biol. 201, 219–227
16. Lu, J., Wiedeman, H., Holmskov, U., Thiel, S., Tinpl, R., and Reid, K. B. M. (1993) Eur. J. Biochem. 215, 793–799
17. Crouch, E., Persson, A., Chang, D., and Heuser, J. (1994) J. Biol. Chem. 269, 17311–17319
18. Malhotra, R., Thiel, S., Reid, K. B. M., and Sim, R. B. (1990) J. Exp. Med. 172, 955–959
19. Malhotra, R., Laursen, B. S., Willis, A. C., and Sim, R. B. (1993) Biochem. J. 293, 15–19
20. Lu, J., Le, Y., Kon, L., Chan, J., and Lee, S. H. (1996) Immunol. 89, 289–294
21. Yee, V. C., Pratt, K. P., Côté, H. C. F., Le Trong, I., Chung, D. W., Davie, E. W., Stenkamp, R. E., and Teller, D. C. (1997) Structure 5, 125–138
22. Aukhil, I., Joshi, P., Yan, Y., and Erickson, H. P. (1995) J. Biol. Chem. 268, 2542–2551
23. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 298–305, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Horsch, M., and Goodman, C. S. (1990) J. Biol. Chem. 265, 15104–15109
25. Fowler, W. E., and Erickson, H. P. (1979) J. Mol. Biol. 134, 241–249
26. Erickson, H. P. (1982) Biophys. J. 37, 96