Microfibril-associated Glycoprotein-1 (MAGP-1) Binds to the Pepsin-resistant Domain of the α3(VI) Chain of Type VI Collagen*

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The interactions of type VI collagen have been investigated, using solid phase binding assays, with two components of the fibrillin-containing microfibrils, the elastin-binding protein, MAGP-1 and its structural relative MAGP-2. Both native and pepsin-treated forms of type VI collagen specifically bound to MAGP-1 but not to MAGP-2. Pepsin type VI collagen was shown to block the binding of MAGP-1 to native type VI collagen indicating that the major MAGP-1-binding site was in the triple-helical region of the molecule. MAGP-1 was found not to bind to collagens I, III, and V. Affinity blotting of pepsin-treated type VI collagen showed that MAGP-1 binding was specific for the collagenous domain of the α3(VI) chain. Decorin and biglycan were found not to inhibit the interaction of pepsin-treated type VI collagen with MAGP-1, indicating that its binding site on the collagen is not close to that for the proteoglycans. Reduction and alkylation of disulfide bonds in MAGP-1 did not destroy its type VI collagen-binding properties, indicating that the binding site was likely to be in the cysteine-free, N-terminal domain of MAGP-1. Interestingly, the interaction of MAGP-1 with type VI collagen was inhibited by tropoelastin, suggesting that the binding sites for tropoelastin and type VI collagen may be in the same domain of MAGP-1. A peptide, corresponding to amino acids 29–38 of MAGP-1, was found to inhibit the interactions of MAGP-1 with type VI collagen and tropoelastin. The results suggest that the peptide may contain the binding sequences for both type VI collagen and tropoelastin, and thus that these two proteins may share the same binding site on MAGP-1. The interactions of MAGP-1 with type VI collagen and tropoelastin were both determined to be of moderately high affinity, with \( K_d \) values of \( 5.8 \times 10^{-7} \) M and \( 2.6 \times 10^{-7} \) M, respectively. The findings indicate that MAGP-1 may mediate a molecular interaction between type VI collagen microfibrils and fibrillin-containing microfibrils, structures which are often found in close proximity to each other in a wide range of extracellular matrices.

Two structurally distinct microfibrillar elements, type VI collagen microfibrils and fibrillin-containing microfibrils, are abundant constituents of the extracellular matrix in a wide range of tissues. Type VI collagen microfibrils (3–5 nm in diameter) are present as an extensive network in virtually all soft connective tissues, where they are found in loose association with collagen fibers and basement membranes, and near the surface of cells (1–7). These microfibrils appear to contain only type VI collagen, present as three distinct α chains, assembled into monomers consisting of a short, central triple-helical region and large globular N-terminal and C-terminal domains (8, 9). The monomers form anti-parallel, disulfide-bonded dimers then tetramers by lateral association. The tetramers are arranged end to end to form the microfibril with a periodic interval of 100 nm (7, 10).

Fibrillin-containing microfibrils are found in association with elastin in elastic fibers, which are prevalent in tissues such as arteries, lung, skin, and elastic ligaments, and as elastin-free bundles in tissues such as kidney, ocular zonule, and spleen (11–17). In contrast to type VI collagen microfibrils, fibrillin-containing microfibrils appear to be complex structures that may contain, or be closely associated with, a number of glycoproteins (17–30) and a chondroitin sulfate proteoglycan (31). Their major structural components are considered to be the fibrillins, which are a two-member family of large (350 kDa), rodlike, cysteine-rich glycoproteins, named fibrillin-1 and fibrillin-2 (17, 19, 25, 26, 29, 30, 32). Parallel arrays of 6–8 fibrillin molecules appear to aggregate, end to end, to form the microfibrils (25, 32). It is uncertain whether the two forms of fibrillin form distinct microfibrils or if they can co-exist within the same microfibril. The fibrillin-containing microfibrils have a characteristic beaded-filament structure, with a 50-nm periodicity, when viewed by the rotary shadowing technique (14, 15, 33). Current evidence suggests that the bundles of fibrillin molecules form the interbead regions, whereas the beads correspond to regions of head to tail interaction between adjacent bundles (25, 32). Evidence suggests that the beads may contain other components including the small structurally related glycoproteins, MAGP-1 and MAGP-2 (25 kDa) (14, 24).

MAGP-1 specifically co-distributes with fibrillin-1 in tissues and has been shown to be localized periodically, at 50-nm intervals, to the beads of the microfibrils (14). MAGP-1 is covalently associated with the microfibrils by disulfide bonding, and it may play a role in the stabilization of the head to tail interaction between fibrillin molecules (14, 20–22). Immunofluorescence staining experiments indicate that the MAGP-1 molecule is accessible on the surface of the microfibril (13), and in vitro binding assays have shown that MAGP-1 will bind to the elastin precursor, tropoelastin (34). These findings suggest that MAGP-1 may participate in the binding and alignment of tropoelastin onto the surface of the microfibrils during elastinogenesis. However, MAGP-1 is also present in microfibrils that do not develop into elastic fibers (16), suggesting that the protein may also be involved in the interaction of the microfibrils with other structural elements of the extracellular matrix.

MAGP-2 is also specifically disulfide-bonded to fibrillin-associ-

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1 The abbreviations used are: MAGP, microfibril-associated glycoprotein; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin.
MAGP-1 and MAGP-2 have been described previously (14, 24). MAGP-2 is associated with microfibrils in tissues, such as nuchal ligament, muscle, spleen, kidney mesangium, and the adventitia of arteries, but is essentially absent from tissues, such as ocular zonule, media of arteries, and the peritubular matrix of kidney. It is possible that MAGP-2 is specifically associated with microfibrils containing only fibrillin-2. The close similarity between MAGP-1 and MAGP-2 is confined to a central region of 60 amino acids where there is precise alignment of 7 cysteine residues. The two glycoproteins are very diverse in the remainder of their structures, suggesting that they may have very distinct functions in microfibril biology (24).

Ultrastructurally, fine microfibrils of type VI collagen are often found in close proximity to the thicker fibrillin-containing microfibrils in a wide range of tissues (2, 16, 35, 36), suggestive of molecular interactions between the two structures. In the present study we have investigated the binding, in vitro, of the microfibrillar proteins MAGP-1 and MAGP-2 with native type VI collagen and the pepsin-resistant fragment of the collagen (pepsin type VI collagen), which corresponds mainly to the triple-helical region. The results indicate that MAGP-1, but not MAGP-2, specifically interacts with type VI collagen. The binding appears to be via a region close to the N terminus of MAGP-1 with a site in or close to the helical region of the α3(VI) chain. The finding provides biochemical evidence that fibrillin-containing microfibrils may indirectly link elastic fibers to collagen fibers via the interaction of MAGP-1 with type VI collagen microfibrils.

**EXPERIMENTAL PROCEDURES**

**Preparation of Macromolecules, Antibodies, and Synthetic Peptides—** MAGP-1, MAGP-2, and MP78/70 (figs-b) were extracted from fetal nuchal ligaments using reducing saline treatment and purified by DEAE-cellulose and gel permeation chromatography as described previously (21), except the proteins were not alkylated and buffers contained 25 mM dithiothreitol. Disulfide bonds were reformed by dialysis into 50 mM Tris buffer, pH 8.0, containing 0.3 M guanidinium chloride, 4 mM cysteine, and 2 mM cystine. Collagen types I, III, V, and VI (native and pepsin-treated) were prepared from fetal calf skin or nuchal ligaments as described previously (37). Tropoelastin was purified using the method of Brown et al. (38), and decorin and biglycan were prepared from the fetal calf by the method of Choi et al. (39). Antibodies to MAGP-1 and MAGP-2 have been described previously (14, 24). For some experiments MAGP-1 was radiolabeled with Na125I (Amersham, Sydney, Australia) using IODO-BEADS (Pierce). Briefly MAGP-1 (95 μg) was dialyzed into 0.1 M phosphate buffer, pH 7.0, containing 8 M urea and reacted with a washed IODO-BEAD and 1 μCi of 125I for 15 min at room temperature. The radiolabeled protein was then dialyzed into 50 mM Tris buffer, pH 8.0, containing 0.3 M guanidinium chloride and stored at −20°C.

**Reduction, Alkylation, and Renaturation of MAGP-1—** For some experiments MAGP-1 was equilibrated in Tris buffer, pH 8.0, containing 4 M guanidinium chloride and 25 mM dithiothreitol. A portion was then alkylated with 55 mM iodoacetamide for 30 min in the dark. Alkylated and nonalkylated aliquots of MAGP-1 were then either directly diluted or dialyzed into 50 mM Tris buffer, pH 8.0, containing 0.3 M guanidinium chloride, 4 mM cysteine, and 2 mM cystine. The samples were then dialyzed further into PBS/milk for the solid phase binding assays (see below).

**Synthetic MAGP-1 peptides** YPDHVQYTHY (peptide 29–38), VIPPTLEPGVTET (peptide 74–87), and QSVAACACCGGC (peptide 170–183), corresponding to amino acids 29–38, 74–87, and 170–183, respectively, of the 183-amino acid sequence of bovine MAGP-1 (22), were a kind gift from Dr. Robert Mecham, Washington University, St Louis, MO. Each peptide was dissolved at a concentration of 20 μg/ml in dimethyl formamide and stored at −70°C under nitrogen.

**Solid Phase Binding Assays—** Plastic flat-bottomed microtiter plates (Nunc-Immuno Maxisorb modules) were coated with different collagen types (usually 1 μg in 200 μl of PBS) at 4°C for 18 h. Control wells were coated with BSA. The wells were then rinsed with PBS, blocked for 30 min with 3% low-fat dried milk in PBS, and washed three times with PBS. MAGP-1 or MAGP-2, either 125I-labeled or unlabeled (0–1 μg in 100 μl of PBS, 0.05% milk), was then added to the wells in quadruplicate, and incubation was continued for 2 h at 37°C. The wells were then washed three times with PBS, 0.05% Tween 20. Binding of 125I-labeled protein to each well was measured directly by scintillation counting. Dissociation constants for interactions were determined using the method of Bidanet et al. (40).

Binding of unlabeled protein was measured using antibody detection with anti-MAGP-1 or anti-MAGP-2 antibody (diluted 1:1000 in PBS/Tween) followed by goat anti-rabbit IgG antibodies conjugated to horse radish peroxidase (1:8000 in PBS/Tween). o-Phenylenediamine was used for color development, which was stopped after 30 min by addition of 0.25 volume of 8 M H2SO4. Color was measured at 490 nm. Controls included omission of protein (MAGP-1 or MAGP-2) from the mobile phase (antibody-only controls).

Blocking experiments were also conducted as described in the appropriate figure legends. These included (a) preincubation of MAGP-1 in the liquid phase with pepsin type VI collagen or tropoelastin (3 μg/μg of MAGP-1) for 2 h at 37°C; (b) incubation of pepsin type VI collagen or BSA-coated wells with decorin, biglycan, MP78/70 or ovalbumin (1 μg/ml) for 1 h at 37°C, prior to the addition of 125I-labeled MAGP-1 in the liquid phase; and (c) addition of synthetic MAGP-1 peptides (25 μg/μg of MAGP-1) to the liquid phase containing MAGP-1 in the mobile phase (antibody-only controls).

**Affinity Blotting—** Duplicate samples (1 μg) of pepsin type VI collagen and MAGP-1 were subjected to SDS-PAGE under reducing conditions on 10% gels and transferred to nylon membranes using previously described methods (21, 37). The membranes were blocked with PBS containing 3% milk for 30 min. One membrane was incubated with MAGP-1 (8 μg/ml) in PBS, 0.05% milk for 18 h, the other was incubated without MAGP-1 as a control. The membranes were then incubated three times with PBS/Tween and incubated with anti-MAGP-1 monoclonal antibody 11B (as ascites diluted 1:500 in PBS/Tween containing 3% milk) for 18 h at 4°C. After rinsing, the membranes were treated for 1 h with goat anti-mouse IgG antibodies conjugated to alkaline phosphatase (Bio-Rad) diluted 1:5000 in PBS, Tween, 3% milk. The membranes were rinsed again, and binding was detected using nitro blue tetrazolium and bromo-chloro-indolylphosphate substrates as described previously (22). After photography, the membranes were counterstained for type VI collagen using polyclonal rabbit anti-(pepsin type VI collagen) antiserum, followed by goat anti-rabbit IgG antibody conjugated to horse radish peroxidase (Bio-Rad). Color was developed using 50 mM Tris buffer, pH 7.6, containing imidazole (10 μM), hydrogen peroxide (0.01%), and diaminobenzidine (600 μg/ml).

**RESULTS**

**MAGP-1 Specifically Binds to Type VI Collagen—** MAGP-1 and MAGP-2 were tested for binding to native type VI collagen using a modified ELISA system (Fig. 1). MAGP-1 showed strong binding that directly correlated with the amount of type VI collagen coated to each well of the microtiter plate. In contrast, MAGP-2 showed no specific interaction with the collagen. To determine if the binding site(s) for MAGP-1 were present in the triple-helical and/or globular domains of type VI collagen, the assay was repeated using pepsin type VI collagen that corresponds to the triple-helical region of the molecule (Fig. 2). Strong binding of MAGP-1 was again observed, indicating that the pepsin-resistant region contains a major binding site. Preincubation of the MAGP-1 with pepsin type VI collagen in the mobile phase greatly reduced the binding to wells coated with pepsin type VI collagen, confirming that the molecular interaction was specific. Interestingly preincubation with pepsin type VI collagen also greatly reduced the binding of MAGP-1 to native type VI collagen. This confirmed that the major MAGP-1-binding site(s) on type VI collagen was in the pepsin-resistant domain.

**MAGP-1 Does Not Bind Collagen Types I, III, and V—** Other collagen types were also tested to determine if the binding of MAGP-1 was specific for type VI collagen rather than for collagens in general (Fig. 3). Radiolabeled MAGP-1 was used in these experiments to avoid variations in the nonspecific bind-

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* M. A. Gibson, J. S. Kumaratilake, and E. G. Cleary, manuscript in preparation.
ing of the anti-MAGP-1 antibodies to wells coated with different collagens. MAGP-1 binding to wells coated with collagen types I, III, or V was very low and was comparable to the levels found in control wells coated with BSA. The binding to type VI collagen was at least 2.5-fold higher than to the other collagen types, indicating that it was specific for this microfibrillar collagen.

MAGP-1 Specifically Binds the α3(VI) Chain of Pepsin Type VI Collagen—Separation of the three α chains of pepsin type VI collagen by reduction and alkylation under denaturing conditions did not eliminate the binding of MAGP-1 to the collagen, provided that the collagen was renatured by dialysis before coating onto the microtiter wells (not shown). This finding suggested that the MAGP-1-binding site(s) was not dependent on the presence of the triple-helical conformation and thus that it was likely to be present on one of the three distinct α chains of the collagen. To identify which chain was involved, the three α chains of pepsin type VI collagen were separated by SDSPAGE under reducing conditions and affinity-blotted with MAGP-1 (Fig. 4). MAGP-1 was found to bind to one band of the pepsin type VI collagen (Fig. 4A), which was not present in the duplicate blot incubated without MAGP-1 but with anti-MAGP-1 antibodies (Fig. 4C). This result indicated that the band was identified by MAGP-1 and not directly by the antibody, thus confirming that the binding was specific. Counterstaining with anti-type VI collagen antibodies (Fig. 4B) showed that the band corresponded to the α3 chain of pepsin type VI collagen, indicating that the MAGP-1-binding site was present on this chain.

Inhibition of the Interaction between MAGP-1 and Type VI Collagen—To determine if the MAGP-1-binding site on type VI collagen could be blocked by decorin or biglycan, the MAGP-1-binding assay was repeated with type VI collagen-coated wells that had been preincubated with each of the above proteoglycans (Fig. 5). Neither macromolecule was found to reduce the binding of MAGP-1 to type VI collagen, suggesting that the proteoglycans bind to a different region of the collagen. MP78/70 was also tested as a potential inhibitor, without effect.

To determine if intact disulfide bonds were important for the interaction with type VI collagen, the binding assay was repeated using MAGP-1 in which these bonds had been irreversibly disrupted by reduction and alkylation under denaturing conditions (Fig. 6). The reduced and alkylated MAGP-1 was found to strongly bind the collagen but only if the protein was slowly renatured by dialysis. This finding indicated that conformation of the binding site is important for activity but that it does not involve disulfide bonds. The result suggested that the type VI collagen-binding site of MAGP-1 was more likely to be in the cysteine-free, N-terminal domain, rather than in the cysteine-rich, C-terminal domain. Since MAGP-1 was known to bind to tropoelastin, the elastin precursor was tested as a potential inhibitor. When MAGP-1 was preincubated with tropoelastin, a major reduction in MAGP-1 binding to type VI collagen was observed.
collagen was observed (Fig. 7), indicating that tropoelastin was specifically inhibiting the interaction. The result suggested that the tropoelastin- and type VI collagen-binding sites on MAGP-1 may be in close proximity to each other. This view was supported by additional experiments in which synthetic peptides corresponding to amino acid sequences of MAGP-1 were added to the binding reaction (Fig. 8). One tyrosine-rich peptide, peptide 29–38, corresponding to the region close to the N terminus of MAGP-1, completely inhibited the interaction of MAGP-1 and type VI collagen. In contrast, two other MAGP-1 peptides had no effect on the binding assay (Fig. 8). The result suggests that peptide 29–38 may compete with MAGP-1 for interaction with type VI collagen and thus that it may correspond to the region of MAGP-1 containing the binding site for the collagen. Interestingly, peptide 29–38 also inhibited the interaction of MAGP-1 with tropoelastin (Fig. 8), suggesting that type VI collagen and tropoelastin may share the same binding site on MAGP-1.

**Measurement of $K_d$ Values for the Interaction of MAGP-1 with Type VI Collagen and Tropoelastin**—To determine the relative affinities of the molecular interactions of MAGP-1 with type VI collagen and tropoelastin, their dissociation constants were measured (Fig. 9). Increasing amounts of $^{125}$I-labeled MAGP-1 were added to wells coated with type VI collagen or BSA. After blocking, the above MAGP-1 samples were added to the wells (0.6 μg/well). Binding was measured by the peroxidase ELISA technique and color development at 490 nm. The binding is expressed as a percentage of the binding of the MAGP-1 sample, which was renatured by dialysis, measured by absorbance at 490 nm. Mean ± S.D. of quadruplicate determinations is shown.

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were calculated using Scatchard analysis. For each MAGP-1 concentration, the ratio of specifically bound counts to unbound counts was plotted against the amount of MAGP-1 bound. Each $K_d$ was calculated from the slope of the resulting straight line which equals $2^{-1/K_d}$ assuming one binding site per molecule (Fig. 9C). The $K_d$ values for the binding of MAGP-1 to type VI collagen and tropoelastin were measured as $5.6 \times 10^{-7}$ M and $2.6 \times 10^{-7}$ M, respectively. This result indicates that MAGP-1 binds more avidly to tropoelastin than to type VI collagen.

FIG. 8. N-terminal peptide 29–38 inhibits MAGP-1 binding to type VI collagen and tropoelastin. Panel A, purified pepsin type VI collagen (solid bars) and BSA (hatched bars) were coated onto microtiter plates (1 μg/well). After blocking, 1.05 × 10^6 dpm of 125I-labeled MAGP-1 (specific activity, 4.2 × 10^5 dpm/μg) was added to each well in the presence or absence of a synthetic MAGP-1 peptide (25 μg/well); see “Experimental Procedures.” Panel B, experiment conducted as in panel A but wells were coated (0.33 μg/well) with tropoelastin (solid bars) and BSA (hatched bars). Column 1, peptide 29–38; column 2, peptide 74–87; column 3, peptide 170–183; column 4, no peptide added. Binding was measured by direct scintillation counting. Mean ± S.D. of quadruplicate determinations is shown.

FIG. 9. Calculation of $K_d$s for interaction of MAGP-1 with type VI collagen and tropoelastin. Panel A, microtiter plates, coated with pepsin type VI collagen (open circles) or BSA (closed circles) (1 μg/well), were incubated with 125I-MAGP-1 at concentrations from 0 to 1 μg/well (specific activity = 2.9 × 10^6 dpm/μg). After a 3-h incubation, the wells were washed, and the amount of bound and unbound radioactivity in each well was determined by scintillation counting. Specific binding (triangles) was calculated as the amount of MAGP-1 bound to type VI collagen-coated wells minus that which bound to BSA-coated wells. Mean ± S.D of quadruplicate determinations. Panel B, a similar experiment as in A except the wells were coated with tropoelastin (open circles) or BSA (closed circles) (250 ng/well). Panel C, data expressed using Scatchard analysis to determine $K_d$ values for MAGP-1 interaction with type VI collagen (triangles) and tropoelastin (squares). Slope of each line = $-1/K_d$.

DISCUSSION

In this study we have demonstrated the specific interaction of type VI collagen, which forms microfibrils 3–5 nm in diameter, with MAGP-1, a component of the fibrillin-containing microfibrils, 10–12 nm in diameter. Type VI collagen has been shown to consist of three genetically distinct polypeptide chains termed α1(VI), α2(VI), and α3(VI), which, like other collagens, form a triple-helical structure (6–8). However, in contrast to most collagens, the molecule has a particularly short triple-helical domain and large N- and C-terminal globular domains. The α3(VI) chain (280–340 kDa) is much larger than the α1(VI) and α2(VI) chains (both 140 kDa), but the sequences forming the triple helix are all virtually identical in length (335–336 residues), and each accounts for about 30 kDa of molecular mass (41, 42). Treatment with pepsin digests the globular domains to yield a resistant fragment consisting of the triple helix plus adjacent regions, containing 2–6 cysteine residues per chain, that form interchain disulfide bonds (8). Analysis of
pepsin type VI collagen by SDS-PAGE yields three bands of approximately 60, 50, and 40 kDa, corresponding to fragments of the α1(VI), α2(VI), and α3(VI) chains, respectively (37, 43).

In the present study, experiments showed that both native type VI collagen isolated predominantly in the tetrameric form, and pepsin type VI collagen bound strongly to MAGP-1. In addition, the binding to both forms of the collagen could be blocked by preincubation of the MAGP-1 with pepsin type VI collagen (Figs. 1 and 2). This finding indicated that the major binding site for MAGP-1 on type VI collagen was located in, or close to, the triple-helical region of the molecule. In similar assays MAGP-1 showed little binding to wells coated with collagen types I, III, and V, indicating that the glycoprotein had a specific, unique interaction with type VI collagen (Fig. 3). It was found that pepsin type VI collagen retained its MAGP-1 binding activity when separated into its constituent polypeptides, indicating that the binding site resided on an individual α chain. Affinity blotting of the three chains of pepsin type VI collagen, separated by electrophoresis, showed that MAGP-1 bound strongly and specifically only to the band corresponding to the α3(VI) chain (Fig. 4). Thus the MAGP-1-binding site appears to be located in the triple helix-forming domain of the α3(VI) chain. It is possible that the site is in the cysteine-rich, helix-flanking regions, but this seems unlikely as these regions are particularly short in the pepsin fragment of the α3(VI) chain (8). In addition the conformations of these flanking regions are likely to have been irreversibly altered by reduction and alkylation of disulfide bonds during chain separation.

A number of other macromolecules have been reported to have specific affinity for type VI collagen. Native type VI collagen has been demonstrated to bind hyaluronan (44, 45). The interaction appears to involve the N-terminal globular domain of the α3(VI) chain (46). Pepsin type VI collagen has been shown to bind collagen types II and XIV, von Willebrand factor, and the dermatan sulfate proteoglycans, decorin and biglycan (40, 47, 48). The binding sequences are not yet known, although decorin and biglycan compete for the same attachment site (40, 47, 48). The findings of the present study indicate that the binding site on MAGP-1 needs further substantiation before the biological implications can be addressed.

Further experiments were conducted to characterize the type VI collagen-binding site on MAGP-1. MAGP-1 is a two domain molecule consisting of an extended, N-terminal domain rich in proline, glutamine, and acidic amino acids, and a C-terminal domain containing all 13 of the cysteine residues and most of the basic residues (22). Since MAGP-1 can be solubilized from fibrillin-containing microfibrils only by disruption of disulfide bonds with a strong reducing agent (20, 21), it is evident that the cysteine-rich, C-terminal domain is involved in the attachment to these structures. MAGP-1 has been shown to bind to tropoelastin in vitro and cell culture experiments indicate that MAGP-1 is important for the deposition of tropoelastin onto the microfibrillar framework during elastic fiber formation (34, 52).

In the present study, reduction and alkylation of MAGP-1, which disrupts the structure of the cysteine-rich, carboxyl-terminal domain, was shown not to inhibit the interaction with type VI collagen (Fig. 6). This finding suggested that the binding site was likely to be found in the cysteine-free N-terminal half of the molecule. Since MAGP-1 also binds tropoelastin (34), its potential inhibitory effect on the interaction of MAGP-1 and type VI collagen was investigated. Preincubation of MAGP-1 with tropoelastin was found to block this interaction (Fig. 7), consistent with the binding sites for type VI collagen and tropoelastin being located in close proximity on MAGP-1. Further inhibition experiments showed that a small synthetic peptide, YPDHVQYTHY, corresponding to amino acids 29–38 of the MAGP-1 sequence, specifically blocked the binding of MAGP-1 to type VI collagen when added to the reaction (Fig. 8A). This tyrosine-rich sequence is close to, but distinct from, the consensus sequence for tyrosine sulfation that involves amino acids 47, 48, and 50 (53). While other explanations are possible, the result suggests that peptide 29–38 is directly competing with MAGP-1 for binding to the collagen. Thus the peptide appears to correspond to at least part of the type VI collagen-binding site. Interestingly peptide 29–38 also inhibited the binding of MAGP-1 to tropoelastin (Fig. 8B), suggesting that type VI collagen and tropoelastin may share the same binding site, close to the N terminus of MAGP-1. Consistent with this idea, MAGP-2, which has a different N-terminal domain to MAGP-1 (24), did not bind type VI collagen (Fig. 1) or tropoelastin (not shown). It should also be noted that the peptide 29–38 sequence overlaps with the recognition sequence (amino acids 21–35) of the anti-(MAGP-1 peptide) antibody recently demonstrated by Brown-Augsburger et al. (52) to inhibit tropoelastin deposition in cell culture. In particular this antibody, when added to culture medium, prevented elastic ear cartilage chondrocytes from organizing newly synthesized tropoelastin into elastic fibers. The distribution of MAGP-1 in the matrix, as detected by an antibody to a different region of the protein, appeared to be normal. These findings led the authors to suggest that the interaction between tropoelastin and fibrillin-containing microfibrils may be mediated by a domain involving the N-terminal half of MAGP-1. The findings of the present study are consistent with this idea.

Scatchard analysis showed that MAGP-1 bound tropoelastin more strongly than type VI collagen (Fig. 9). This suggests that, if the two proteins do share the same binding site on MAGP-1, the recognition sequences are unlikely to be identical. Comparison of the amino acid sequences of the tropoelastin and the triple helix-forming region of the α3(VI) chain revealed no regions of close similarity that might represent a binding-consensus sequence. However, both proteins contain many glycine and proline residues, and it is possible that MAGP-1 recognizes specific sequences rich in these amino acids. The results also suggest that tropoelastin may have the potential to displace bound type VI collagen from MAGP-1. However, the possibility that tropoelastin and type VI collagen share the same binding site on MAGP-1 needs further substantiation before the biological implications can be addressed.

The binding affinity between individual MAGP-1 and type VI collagen molecules was found to be of moderately high strength (Fig. 9) and was of a similar order to the interaction between type VI collagen and decorin (40). However, the potential interaction between the two types of microfibril may be much stronger due to the possibility for multiple interactions between individual microfibrils. Potentially there are two pairs of

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3 M. L. Finnis and M. A. Gibson, unpublished observations.
MAGP-1-binding sites present per tetramer (or 100-nm interval), along each type VI collagen microfibril that may interact with multiple MAGP-1 molecules attached to each bead (or 50-nm interval), of the fibrillin-containing microfibrils. However, there is no ultrastructural evidence for a regular lateral alignment or linear overlap of the two types of microfibril in tissues. This suggests that some sites may be blocked by other matrix molecules or that the binding sites on the type VI collagen microfibrils are not equally spaced with the MAGP-1 molecules along the fibrillin-containing microfibrils, resulting in a less ordered pattern of interaction.

Overall the above findings support the concept that MAGP-1 is anchored to the surface of, or within, the beads regions of the fibrillin-containing microfibrils by its C-terminal domain and that its N-terminal domain is available for interactions with macromolecules of the surrounding matrix. The results provide evidence for the binding of MAGP-1 to the triple-helical domain of the α3(VI) chain, and thus for the possibility of molecular interactions of fibrillin-containing microfibrils with type VI collagen microfibrils in tissues. The findings support ultrastructural observations of a close physical relationship between fibrillin-containing microfibrils of elastic fibers and the finer, collagen fiber-associated, type VI collagen microfibrils in tissues such as ligament and aorta (11, 51, 54). These elastin-associated microfibrils are considered to provide an anchoring function for the elastic fiber during stretching in tissues such as nuchal ligament, lung, and aorta (12). While anchorage may be provided by the interaction of the microfibrils with components of basement membranes in some tissues, this cannot be the case in tissue such as nuchal ligament, which lacks the latter structures. The results suggest that the elastin-associated microfibrils also have the potential to be anchored indirectly to collagen fibers via type VI collagen microfibrils. The interaction of MAGP-1 and type VI collagen may also be important for the tethering of bundles of fibrillin-containing microfibrils in some nonelastic tissues that are rich in type VI collagen, such as kidney mesangial matrix (5).

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