A Single Chain 19-kDa Fragment from Bovine Thrombospondin Binds to Type V Collagen and Heparin*

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Location of the type V collagen-binding domain within bovine thrombospondin (TSP) was investigated by using fragments of reduced and alkylated TSP. A fragment of relative molecular mass (19 kDa) was isolated, which inhibited binding of 125I-TSP to type V collagen in a solid-phase binding assay. A direct binding assay using the 125I-labeled fragment confirmed that the fragment actually bound to collagen. The fragment retained specificity for the native structure of type V collagen like the intact TSP molecule. Its binding to the collagen, however, was not inhibited by Ca2+ in contrast to intact TSP. Amino acid sequence analysis of the 19-kDa fragment suggested that this fragment corresponded to Val420-Lys457, a part of the stalklike region in the human TSP primary structure. It was found that the fragment also bound well to heparin in a specific and saturable manner and, furthermore, binding to type V collagen was inhibited by soluble heparin. Removal of the N-linked sugar chain from this fragment resulted in a 14-kDa fragment. The deglycosylated fragment retained the ability to bind to type V collagen as well as heparin. These results suggest that a type V collagen-binding site is present in the 80-residue portion of bovine TSP (Val420-Lys457) and is likely to be identical or lie very close to a heparin-binding site, which exists in the type I repeat structure.

Thrombospondin (TSP)† is a glycoprotein of a large molecular mass (about 450 kDa) and is composed of three identical polypeptide chains that are held to one another with disulfide bonds (1). It was first identified as a protein that is secreted from the α-granules of thrombin-activated platelets, though it is now well known to be secreted by a variety of cells and may be a transient component of the extracellular matrix (2, 3). Apparently, TSP is a multifunctional protein: it supports aggregation of platelets (4), mediates attachment and spreading of several cell types (5, 6), promotes motility of human neutrophils (7) and tumor metastasis (8), and is essential for smooth muscle cell proliferation (9). An antiangiogenic activity of TSP has also been proposed (10, 11).

The functions of TSP described above are ascribed to its ability to interact with many components of the extracellular matrix as well as cell surface receptors. TSP can bind, for example, heparin (12), laminin (13), fibrinogen (14), fibronecin (15), plasminogen (16), and transforming growth factor-β (17). In addition to these ligands, TSP is known to bind to collagen with interesting specificity; it binds preferentially to type V collagen (13, 18). The location of the TSP domains responsible for the interaction with these molecules has been actively investigated by many workers. The amino-terminal 25-kDa small globular domain mediates adhesion and migration of Chinese hamster ovary cells by binding to cell-surface heparan sulfate proteoglycans (19). The COOH-terminal large globular domain contains not only an RGDA sequence that mediates integrin-directed cell attachment (6) but also another cell attachment site (20). The central stalklike domain is another well known domain that contains binding sites for many ligands. From this region, a 210-kDa core fragment composed of three identical polypeptides of 70 kDa was obtained by limited digestion with chymotrypsin (21). This fragment is known to contain the binding site for type V collagen, which has rather unique characteristics among the fibril-forming collagens.

We have been investigating another collagen-binding glycoprotein, the propolypeptide of von Willebrand factor (22, 23), which specifically interacts with native type I collagen fibers but not with type V (24). We have determined the location of its collagen-binding domain (25, 26) and have obtained a collagen-binding peptide of 10 residues (27). It is of interest to know whether the collagen-binding sites in different collagen-binding proteins are structurally related to one another or are completely different. To attempt this comparison, we must obtain information about the collagen-binding sites of various proteins.

In the present work, we tried to narrow down the type V collagen-binding site of bovine TSP and obtained a 19-kDa fragment capable of binding to type V collagen.

EXPERIMENTAL PROCEDURES

Materials—Bovine type I-V collagens solubilized by pepsin were purchased from Koken Ltd. (Tokyo, Japan). Lysyl endopeptidase was from Wako Pure Chemical Co. (Osaka, Japan). Endo-β-N-acetylgalactosaminidase F (Endo F) and peptide N-glycosidase F were from Boehringer Mannheim. All other chemicals were of analytical grade.

Purification of TSP—Bovine TSP was purified from calcium ionopore A23187-treated platelets by using heparin-Sepharose chromatography in a slight modification of the method of Lawler et al. (21). Supernatant from A23187-treated platelets was applied to an Affi-Gel gelatin (Bio-Rad) column to remove fibronectin, and the run-through fraction was consecutively applied to a heparin-Sepharose CL-4B column (Pharmacia LKB Biotechnology Inc.). The fractions eluted with 0.25–0.55 M NaCl were pooled and applied to a column of Sephacryl S-300 (2.5 × 90 cm) (Pharmacia) to remove low
molecular weight proteins. The concentration of TSP was determined by using the extinction coefficient ($E_{280}^1 = 10.9$) reported by Margossian et al. (28).

**Generation and Characterization of Proteolytic Fragments of TSP**—To achieve complete digestion, TSP was S-acetamidomethylated with iodoacetamide before treatment with lysyl endopeptidase according to the method described previously (26). Five milligrams of lyophilized S-acetamidomethylated TSP (Acm-TSP) were dissolved in 15 mM Tris-HCl, pH 9.0, containing 4 M urea, and lysyl endopeptidase was added at an enzyme/substrate ratio of 1:200. After incubation at 30 °C for 6 h, the mixture was passed through a column of Econo-Pac 100D, and the desalted protein fraction was lyophilized, dissolved in 200 µl of 0.2 mM ammonium acetate, pH 4.8, and subjected to size-exclusion high performance liquid chromatography (HPLC) on a TSK-gel G3000SWxL column (0.78 × 30 cm, Tosoh Co., Tokyo, Japan) equilibrated with the same buffer using a Waters 625 LC system. Each fraction was assayed for inhibition of collagen binding, and the fraction containing inhibitory activity (fraction A) was applied to anion-exchange HPLC on a Shodex IEC QA-825 column (0.8 mm × 32 cm, Showa Denko Co., Tokyo, Japan) equilibrated with 20 mM Tris-HCl, pH 8.0. The column was washed with the equilibration buffer and eluted with a linear gradient of NaCl (0-500 mM). Pooled fractions were dried and dissolved in H2O and subjected to the collagen binding inhibition assay.

**Radioiodination of Proteins**—Radio labeling of TSP and the 19-kDa fragment was achieved either with Na[125I] (Amersham Corp.) and IODO-Beads (Pierce Chemical Co.) or with [35S]-Bolton hunter radiolabel (Amersham) according to the manufacturer's recommendations (27). About 1-10 µg of protein were routinely labeled in each experiment, and the specific activities ranged between 70 and 260 MBq/mg protein.

**Binding Inhibition Assay**—The collagen binding properties of TSP or the 19-kDa fragment were assessed using a solid-phase binding assay. Each well of a detachable 96-well plate (Breakable Combiplate 8, Labsystems, Helsinki, Finland) was coated with 2.5 µg of collagen in 50 µl of TBS (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) by overnight incubation at 4 °C. The amount of the protein adsorbed to the surface of the wells determined using a biocinchonic acid protein assay kit (Pierce, Co., Rockford, IL) was 5.5 mM NADH and 0.05% SDS, and it was confirmed that about 30-40% of the protein was adsorbed to the wells regardless of the differences in collagen preparations. The nonspecific protein-binding sites were blocked with TBS containing 1% bovine serum albumin (BSA/TBS) for more than 1 h at room temperature. Forty microliters of 125I-labeled proteins in BSA/TBS were added in duplicate and incubated for 90 min at room temperature together with competing reagents to be tested. After being washed three times with cold TBS, the plate was divided into separate wells, and the radioactivity of bound ligand was counted on a γ counter. The value obtained with wells that had not been coated with collagen was subtracted as it represented nonspecific binding. Binding to heparin was measured by essentially the same method used for collagen binding except that the wells were coated with heparin-BSA conjugate prepared as described (29).

**Deglycosylation of Proteins**—A purified 19-kDa fragment was treated with 1 unit/ml Endo F and 4 units/ml peptide N-glycosidase F in TBS for 15 h at 37 °C and analyzed by SDS-PAGE by the method of Laemmli (30). The [35S]labeled 19-kDa fragment was also deglycosylated as above and subjected to collagen binding assay. After washing the well, the bound protein was eluted with SDS sample buffer and analyzed by SDS-PAGE and autoradiography.

**Amino Acid Sequence Analysis**—The amino acid sequence of the 19-kDa fragment was determined as follows. About 30 µg of purified 19-kDa fragment were dissolved in 50 mM phosphate buffer, pH 7.2, containing 10 mM EDTA and digested with L-1-lysylamidol-2-phenylethyl chymotrypsin at an E/S ratio of 1:50 for 24 h at 37 °C. Peptides generated by this complete digestion were separated by reverse-phase HPLC on an Ultrasphere ODS column (0.46 × 25 cm, Beckman) using a linear gradient of acetonitrile and subjected to amino acid sequence analysis on an automated Gas-Phase Sequenator (model 470A, Applied Biosystems). Identifications were followed by identification of phenylthiohydantoin derivatives by on-line HPLC (model 120A, Applied Biosystems).

**Results**

It is well known that human platelet TSP binds specifically to type V collagen among the various types of collagen, though much lower but significant levels of binding are also observed in types I-IV collagens. As we used bovine platelet TSP as the material to determine the collagen-binding domain of TSP in this study, we first investigated the properties of the binding of bovine TSP to various types of collagen. As is shown in Table 1, the amount of bovine TSP bound to type V collagen was by far the highest. The binding was dependent on the native structure of the collagen since TSP could not bind to heat-denatured type V collagen. The amount of collagens actually coated on the surface of the wells was determined by protein assay, and approximately the same values were obtained for different collagen preparations. We also confirmed that the bound collagens did not detach from the wells during incubation with the buffer for 90 min at room temperature, i.e. the same condition as that employed in the assessment of TSP binding. This excludes the possibility that the difference in the binding was due to lower coating efficiency of the type I-IV or denatured collagens. In the presence of 2 mM Ca++, the binding to type V collagen was reduced to about 25% of that observed in the absence of Ca++ (data not shown). These results indicate that bovine TSP has the same collagen binding properties as human TSP; it binds fairly specifically to native type V collagen, and the binding is inhibited by Ca++.

Using bovine TSP as a starting material, we tried to obtain protease fragments capable of binding to type V collagen. Before protease digestion, bovine TSP was reduced and alkylated with iodoacetamide to unravel the rigid globular structure. Interestingly, reduction and alkylation had no effect on the binding affinity of TSP to type V collagen since Acm-TSP inhibited binding of 125I-TSP to type V collagen with almost the same dose dependence as native TSP (data not shown). This Acm-TSP was then treated with lysyl endopeptidase in the presence of 4 M urea, and complete digestion was achieved. The Acm-TSP produced numerous fragments of relative molecular mass less than 20 kDa, and the digest was subjected to size-exclusion HPLC (Fig. 1). Several fractions were collected and analyzed by SDS-PAGE (Fig. 1, inset). Fraction A contained only one diffuse band of average molecular mass of about 20 kDa on SDS-PAGE. Fractions B-D comprised a mixture of peptides of 17, 14, 8, and 6.5 kDa as major components. No obvious band was observed in lane E (fraction E) indicating that this fraction contained small peptides that could not be detected by the standard Coomassie staining technique. We examined the inhibitory activities of these fractions (A-D) on the binding of 125I-TSP to type V collagen using about equal concentrations. Significant inhibitory activity was observed only with fraction A (Fig. 2). This result strongly indicates that fraction A contains a fragment that can compete with intact TSP upon binding to type V collagen. The collagen-binding fragment was further purified from fraction A by using anion-exchange chromatography on a Shodex QA-825 column (Fig. 3). Fraction A contained several distinct fragments of a relative molecular mass of 19-20 kDa. Among these fragments, a 19-kDa fragment that comprises 125I-TSP binding to type V collagen when added at the same concentration (Fig. 4). This inhibition by the purified 19-kDa

<table>
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<th>Protein coated</th>
<th>Bound counts/min ± S.D.</th>
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<tr>
<td>BSA</td>
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<td>5961 ± 18</td>
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<td>Type V collagen (heat denatured)</td>
<td>856 ± 15</td>
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A Collagen-Heparin-Binding Fragment from Thrombospondin

To confirm that the 19-kDa fragment is really a collagen-binding fragment, the fragment was labeled with 125I, and its binding to type V collagen was directly assessed. As expected, the 125I-labeled 19-kDa fragment showed specific binding to type V collagen (Fig. 6). The binding was saturable, and the addition of a 200-fold excess of unlabeled 19-kDa fragment completely abolished the apparent binding of the 125I-labeled 19-kDa fragment. Furthermore, the specificity of collagen recognition by the 19-kDa fragment was very similar to that of intact TSP in that it preferentially bound to type V collagen among type I–V and did not bind to heat-denatured type V collagen (data not shown). As to the sensitivity to calcium ion, however, the collagen binding behavior of the 19-kDa fragment was totally different from that of intact TSP. Binding of the 19-kDa fragment to type V collagen was not affected at all by addition of Ca2+ up to 5 mM, while the binding of intact TSP was greatly diminished by Ca2+ at concentrations higher than 200 μM (Fig. 7).

To determine the location of the 19-kDa fragment in the TSP molecule, the fragment was further digested by trypsin. Several peptides were obtained from the digest used reverse-phase chromatography and subjected to amino acid sequence analysis. Assuming sequence similarity between bovine and human TSP, these sequences were aligned according to that of human TSP predicted from the cDNA sequence (31). All peptides identified had a corresponding sequence in the region between Val333 and Lys412 of human TSP, and the COOH-terminal lysine residue was found in peptide 5 (Fig. 8). In fact, all the identified residues (57 amino acids) were completely conserved between the bovine and human sequences. It seems obvious that the type V collagen-binding 19-kDa fragment we observed corresponds to Val333 to Lys412 in the bovine TSP primary structure, which is included in the 210-kDa trimeric fragment previously reported as the collagen-binding domain (21). None of the peptides obtained by trypsin digestion of the 19-kDa fragment showed a significant inhibitory effect on collagen binding at the concentration tested (about 1 μM at the highest, data not shown). Whether this lack of inhibition is due to cleavage of a collagen-binding site by the second digestion or insufficient peptide added in the assay system is not clear.

Within the region Val333 to Lys412, there was one possible N-linked carbohydrate attachment site (Asn342). Deglycosylation of the 19-kDa fragment with Endo F and peptide N-glycosidase F clearly showed that the fragment did have an N-linked sugar chain (Fig. 9A). The relative molecular mass of the deglycosylated fragment was about 14 kDa, which was higher than the calculated molecular mass of 9488 for Val333 to Lys412. The reason for the discrepancy between the calculated and observed values for the deglycosylated polypeptide is not clear. Further treatment of the 14-kDa fragment with sialidase and O-glycosidase did not result in any bands with reduced molecular mass (data not shown), suggesting that the fragment is devoid of O-linked sugar chains. However, it is still possible that it contains unique carbohydrate chain(s) resistant to the enzymes used in this study. The effect of deglycosylation of the 19-kDa fragment on its binding to type V collagen was also investigated. The 125I-labeled 19-kDa fragment was treated either with or without Endo F/peptide N-glycosidase F and allowed to bind to type V collagen-coated wells. After washing with buffer, the bound fragment was solubilized with SDS and analyzed by SDS-PAGE. As shown in Fig. 9B, the deglycosylated form of the 19-kDa fragment (i.e. 14-kDa fragment) even exhibited increased binding compared with the intact 19-kDa fragment. This result ruled out the possibility of participation of N-linked carbohydrate moieties in collagen recognition.

The complete matching of the sequence of the 19-kDa fragment, as far as it was elucidated, with the human sequence Val333 to Lys412 strongly suggests that it contains one possible heparin-binding sequence (WSXW), which has been reported recently by Guo et al. (32). We investigated whether the 19-kDa fragment specifically interacts with heparin by using heparin-conjugated BSA. The 19-kDa fragment did bind to
FIG. 3. Purification of a 19-kDa collagen-binding fragment by anion exchange chromatography on Shodex QAE. Fraction A from size exclusion chromatography was applied to a Shodex IEC QA-825 column and separated by a linear gradient of NaCl. Fractions indicated by the bold horizontal bars (1-5) were pooled and subjected to analysis by SDS-PAGE using a 15% gel followed by staining with Coomassie Brilliant Blue (inset). The positions of molecular size markers are indicated on the left.

FIG. 4. Effect of fractions separated by anion-exchange chromatography on TSP binding to type V collagen. Methods were essentially the same as described in the legend to Fig. 2 except that the fractions were added at 1 μg/well.

FIG. 5. Dose-dependent inhibition of TSP binding to type V collagen by the 19-kDa fragment. Purified 19-kDa fragment (closed circles) or intact TSP (open circles) was added to the reaction mixture at the concentrations indicated. The experimental conditions were the same as those described in the legend to Fig. 2.

FIG. 6. Specific binding of 125I-labeled 19-kDa fragment to type V collagen immobilized on a microtiter well. Increasing amounts of the 19-kDa fragment were added in a total volume of 40 μl. The total amounts of 19-kDa fragment bound were calculated from the specific activity.

FIG. 7. Effect of Ca2+ concentration on the binding of TSP and 19-kDa fragment to type V collagen. Bindings are expressed as percent of that obtained in the presence of 2 mM EDTA. Open circles, TSP binding; closed circles, 19-kDa fragment binding.
peptides from the 19-kDa fragment were isolated by reversed-phase HPLC and subjected to amino acid sequence analysis. The obtained peptide sequences were aligned considering similarity in the human segment but not identified in the bovine fragment. The residue that are conserved between the human and bovine proteins are boxed.

Deglycosylation of the 19-kDa fragment. A, purified 19-kDa fragment was treated with (+) or without (−) the mixture of Endo F and peptide N-glycosidase F (PNGase F) at 37 °C for 15 h and analyzed by SDS-PAGE using a 15% gel followed by Coomassie Brilliant Blue staining. B, 125I-labeled 19-kDa fragment was treated with (+) or without (−) Endo F/peptide N-glycosidase F as described above and added to wells coated with type V collagen. Fragments bound to each well were eluted and subjected to SDS-PAGE on 15% gel followed by autoradiography.

Table II

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<th>Protein coated</th>
<th>Heparin added</th>
<th>Bound counts/min ± S.D.</th>
<th>μg/ml</th>
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<tr>
<td>Unconjugated BSA</td>
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<td>198 ± 21</td>
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<tr>
<td>Phosphotyrosine-BSA</td>
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<td>279 ± 32</td>
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<tr>
<td>Heparin-BSA</td>
<td>0</td>
<td>1843 ± 133</td>
<td>100</td>
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Table III

<table>
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<th>Heparin concentration</th>
<th>TSP binding</th>
<th>19-kDa binding</th>
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<tr>
<td>μg/ml</td>
<td>μg/well</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>1798</td>
<td>92.9</td>
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<tr>
<td>10</td>
<td>64.3</td>
<td>3.5</td>
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DISCUSSION

The study presented here provides evidence that the type V collagen-binding site of TSP is located in a region between Val133 and Lys412. The collagen binding behavior of a fragment corresponding to this region is quite similar to that of the whole TSP molecule except for the effect exerted by Ca²⁺. Apparently, the association with Ca²⁺ changes the configuration of TSP and inhibits its binding to collagen, as suggested previously (18). The lack of inhibition by Ca²⁺ in the 19-kDa fragment binding to type V collagen suggests that it contains the collagen-binding site(s) but not the Ca²⁺-binding domain. However, it is still possible that a Ca²⁺-sensitive collagen-binding site exists in the native TSP molecule and became undetectable due to lysyl endopeptidase digestion. Experiments using other proteases with different specificities may clarify this point. Until now, the minimum segment known to represent the type V collagen-binding site of TSP was the 210-kDa trimeric fragment originating in the central stalklike region. This fragment, obtained by mild digestion with chymotrypsin, is comprised of three identical polypeptides of 70 kDa cross-linked by disulfide bonds (21). The NH₂-terminal residue of the fragment is Ile241, while the COOH-terminal residue has not been identified. This region contains numerous cysteine residues, and it is obvious that they form intrapolyptide disulfide bridges in addition to intersubunit cross-linking at Cys352 and Cys354 (33). In contrast, the 19-kDa fragment we have obtained is derived from S-acetylated TSP and has no disulfide-bonded cysteine, indicating that this fragment may not retain the intact tertiary structure of the original protein. As the apparent size of the purified 19-kDa fragment estimated by size-exclusion chromatography was about 15–20 kDa, this fragment exists as a monomer but not as noncovalent oligomers, at least under the conditions employed here. Thus it seems possible that the linear sequence of a segment in this fragment serves as a collagen-binding site. We could not narrow down the binding site further, because none of the peptides obtained by trypsin digestion of the 19-kDa fragment showed significant inhibitory effects on collagen and heparin binding at the concentrations tested. It remains a possibility that the affinity of the binding sites became so low that we could not detect them under our experimental conditions. More rigorous experiments using various synthetic peptides remain to be conducted to elucidate the precise location of the binding site.

The 19-kDa fragment comprises the COOH-terminal third portion of the procollagen-like domain and the first copy of the type I repeat structure. The type I repeat structure has recently emerged as a very interesting domain. One of the interesting features of the type I repeat structure reported recently by Guo et al. (32) is that it has a unique heparin binding sequence WSXW. Our observation that the 19-kDa heparin in a specific and saturable manner (Table II). The binding was strongly inhibited by the addition of excess soluble heparin and was not observed with either unconjugated BSA or with BSA conjugated with phosphotyrosine. Removal of the N-linked sugar chain did not reduce the binding of the fragment to heparin either (data not shown). The fact that the 19-kDa fragment binds both to type V collagen and to heparin enables us to speculate that the binding site for these two ligands may be related. As expected, the binding of bovine TSP as well as the binding of the 19-kDa fragment to type V collagen were strongly inhibited by heparin (Table III). The IC₅₀ values of heparin for these bindings were almost the same (~0.5 μg/ml), and more than 90% inhibition was achieved with a concentration of 10 μg/ml.
fragment, which is likely to contain the sequence WSEE, bound to heparin and consistent with their observation. Before Guo’s report (32), it was believed that the 25-kDa fragment derived from the NH₂-terminal end of TSP was the heparin-binding domain. Though this heparin-binding fragment has no collagen binding activity, Galvin et al. (18) reported that binding of human TSP to type V collagen was inhibited by heparin. This contradiction seems to be resolved if one assumes that there is a second heparin-binding site close to the collagen-binding site in TSP. Our observation that the binding of the 19-kDa fragment to type V collagen is diminished by heparin in fact suggest that the heparin-binding site (WSXW) is the type V collagen-binding site itself or that they are very close to each other.

Another well known characteristic of the type I repeat is that it contains three homologous copies of the consensus sequence W(S)(R)CSVTCG, which is also present in collagen and many circumsarcozoaote proteins (34). The VTCG sequence in malarial circumsarcozoaote protein was found to have cell attachment activity (35). Frater et al. (36) reported that a peptide containing this sequence promoted melanoma cell adhesion, and an antibody directed against this peptide inhibited cell adhesion to TSP. Tuszyński et al. (37) also reported that peptides with CSVTSC or CSTSCG sequences inhibited tumor cell metastasis and platelet aggregation. As the 19-kDa fragment we have obtained has the sequence WSEWTSCSTSCG, it may have the adhesive property described above. This point also needs to be investigated.

The 210-kDa core fragment derived from the central stalk-like region is known to contain a binding site not only for type V collagen but also various ligands such as laminin, fibrinogen, and plasminogen (21). In a preliminary experiment, the 19-kDa fragment did not bind to fibrinogen and laminin at all and bound only slightly to plasminogen. However, the binding of intact TSP to plasminogen was not affected by heparin, suggesting that the binding sites for these ligands are different from the collagen-binding site. The structural relationship among binding sites to these ligands remains to be clarified.

Type V collagen is widely distributed as a minor component of the extracellular matrix in various tissues (38). It is thought to make complexes with more abundant type I collagen form heterotypic fibers and regulate the diameter of these fibers (39). Type V collagen has several unique characteristics compared with other collagens; it binds to DNA (40), heparin (41), insulin (42), and of course TSP. Furthermore, type V collagen is a substrate for glycosaminoglycan-mediated cell attachment that can be inhibited by soluble heparin or heparan sulfate (43), while cell adhesion to other types of collagen is thought to be mainly mediated by VLA2 integrin (44). Though the physiologic importance of the specific binding of TSP to type V collagen is not clear, these two proteins may be involved in organogenesis and tissue morphogenesis by modulating their ability to bind to cells. The complete primary structure of the human collagen α1(V) chain has recently been determined (45) and shown to have a unique NH₂-terminal noncollagenous region that is much larger than those of other collagens. It may be possible that TSP binds to type V collagen through this unique region. Determination of type V collagen structures responsible for binding to TSP and heparin is currently under investigation.

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REFERENCES


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