Altered Metabolism of Thrombospondin by Chinese Hamster Ovary Cells Defective in Glycosaminoglycan Synthesis*

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We examined the ability of Chinese hamster ovary (CHO) cell mutants defective in glycosaminoglycan synthesis to metabolize \(^{125}\)I-labeled thrombospondin (TSP). Wild type CHO cells bound and degraded \(^{125}\)I-TSP with kinetics similar to those reported for endothelial cells. Both binding and degradation were saturable (half-saturation at 20 µg/ml). When the concentration of labeled TSP was 1–5 µg/ml, mutant 745, defective in xylosyltransferase, and mutant 761, defective in galactosyltransferase I, bound and degraded 6- to 16-fold less TSP than wild type; mutant 803, which specifically lacks heparan sulfate chains, bound and degraded 5-fold less TSP than wild type; and mutant 677, which lacks heparan sulfate and has increased levels of chondroitin sulfate, bound and degraded 2-fold less TSP than wild type. Binding and degradation of TSP by the mutants were not saturable at TSP concentrations up to 100 µg/ml. Bound TSP was localized by immunofluorescence to punctate structures on wild type and, to a lesser extent, 677 cells. Heparitinase pretreatment of wild type cells caused a 2- to 3-fold decrease in binding and degradation, whereas chondroitinase pretreatment had no effect. Chondroitinase pretreatment of the 677 mutant (deficient in heparan sulfate and excess chondroitin sulfate) caused a 2-fold decrease in binding and an 8-fold decrease in turnover, whereas heparitinase pretreatment had no effect. Treatment of wild type cells with heparitinase and chondroitinase resulted in a 6- to 8-fold decrease in binding and turnover. These results indicate that cell surface proteoglycans mediate metabolism of TSP by CHO cells and that the primary effectors of TSP metabolism are heparan sulfate proteoglycans.

Thrombospondin (TSP) is a large (450,000 dalton) heparin-binding glycoprotein composed of three apparently identical subunits (1-4). TSP is a major macromolecule released from the α granules of stimulated platelets (5, 6). It is synthesized by and is present in the extracellular matrices of a variety of cell types in vitro: endothelial cells (7-9); aortic smooth muscle cells (9); fibroblasts (10); granular type II pneumocytes (11); keratinocytes (12); and glial cells (13). The functional significance of TSP in hemostatic events has been the focus of numerous studies (reviewed in Refs. 14 and 15). However, the effects of TSP on cellular behavior are equally interesting. Synthesis of TSP by cultured cells is modulated by factors which affect the cell cycle, such as cell density (16) and growth factors (platelet-derived growth factor) (13, 17), and there is evidence that TSP is an autocrine modulator of proliferation of cultured smooth muscle cells (18). By immunolocalization techniques, TSP is more prevalent in wounded or developing tissues than in differentiated, adult tissues (19, 20). These findings suggest that TSP may be an important mediator of tissue growth and/or remodeling.

Several different molecules may serve as receptors for binding of TSP to cells. Roberts et al. (21, 22) showed that TSP binds to sulfatides on endothocyte membranes and mediates cytadherence of Plasmodium falciparum-infected erythrocytes. TSP has also been reported to mediate attachment and spreading of a number of cell types, including certain strains of melanoma cells and squamous cell carcinoma cells (23-25). Asch et al. (26) demonstrated saturable binding of TSP to melanoma and HT 1080 fibrosarcoma cells and presented evidence that the receptor on these cells is the 88,000-dalton protein recognized by the OKM5 monoclonal antibody. We have described pathways for the metabolism of TSP by bovine aortic endothelial cells and human fibroblasts in which cell surface receptors mediate endocytosis and degradation of TSP (27, 28). Binding and degradation of labeled TSP to endothelial cells was inhibited by heparin and ficollan, which apparently compete for the amino-terminal heparin-binding domain of TSP and by platelet factor 4, β-thromboglobulin, and whole blood serum which may compete for similar endothelial cell surface-binding sites (28). Binding of TSP to endothelial cells was decreased by pretreatment of cells with trypsin or heparitinase but not by pretreatment with chondroitinase or hyaluronidase.

Proteoglycans intercalated into cell membranes may function as receptors for different macromolecules, including antithrombin III (29), collagen (30), lipoprotein lipase (31), and platelet factor 4 (32). Based on our results with the bovine endothelial cells, we hypothesized that cell surface proteoglycans mediate binding and degradation of TSP by cells (28). To test this hypothesis, we studied the interactions of TSP with a series of Chinese hamster ovary (CHO) cell mutants that are defective in various stages of glycosaminoglycans.

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‡The abbreviations used are: TSP, thrombospondin; BSA, bovine serum albumin; CHO, Chinese hamster ovary; LDL, low density lipoprotein; PBS, phosphate-buffered saline.
biosynthesis (33–35). These mutants are also deficient in trypsin-releasable cell surface proteoglycans (Ref. 36 for mutants 745 and 761). We found that TSP interacts with specific and saturable binding sites on wild type CHO cells. This interaction has been characterized and to that reported for bovine thrombomodulin and endothelial cells (28). Binding and degradation of TSP by mutant cell strains that lack cell surface proteoglycans were reduced. Thus, these investigations support the hypothesis that cell surface proteoglycans mediate the binding and degradation of TSP and suggest a role for cell surface proteoglycans in a receptor-mediated endocytic process.

MATERIALS AND METHODS

Mutant CHO Cells—Chinese hamster ovary cells (CHO-K1), originally obtained from the American Type Culture Collection (CCL-61), were cultured in Ham’s F-12 medium containing 10% fetal bovine serum (Hyclone, Logan, UT) without antibiotics. The development end characterization of the mutant CHO cells has been described in detail elsewhere (33–35). Wild type cells have an 8:1 ratio of heparan sulfate to chondroitin sulfate cell surface proteoglycans. The properties of the mutant strains used in the present experiments are summarized in Table I.

Thrombospondin Purification and Labeling—TSP was isolated, as described previously, from the release products of thrombin-stimulated human platelets by heparin-affinity and gel-filtration chromatography (37). TSP was labeled with 125I by the chloramine-T method, followed by repurification on a heparin affinity column (27). Preparations were greater than 98% precipitable by 10% trichloroacetic acid, and nonreduced and reduced labeled protein migrated as single bands when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

Assays of Binding and Degradation—Confluent cultures (35-mm dishes) were incubated with mixtures of unlabeled and 35S-labeled TSP in Ham’s F-12 medium with 0.2% BSA (F-12/BSA). After the incubation period, binding medium was removed, and the wells were washed three times with cold F-12/BSA. Bound TSP was assayed by counting the radioactivity in the washed cell layer solubilized with 1 N sodium hydroxide. In some assays, nonspecific binding was determined in the presence of 1 μg/ml heparin (Sigma, porcine intestinal mucosa) (28). Assays to determine saturability of binding were performed at 4 °C with a 2-h incubation. Degradation of 125I-labeled TSP was determined by quantification of radioactive labeling of the binding medium that was soluble in 10% trichloroacetic acid at 1 and 4 h of incubation at 37 °C and subtraction of the 1-h value from the 4-h value. All assays were performed in duplicate on at least two separate occasions.

Enzymatic Pretreatments—Cell monolayers in 35-mm dishes were incubated for 3 h at 37 °C with 1 ml of F-12/BSA containing 1 unit of heparitinase from Flavobacterium heparinum or 0.5 unit of chondroitinase ABC from Proteus vulgaris or both enzymes together (Miles Laboratories, Elkhart, IN). After this pretreatment, 2.5 μg of 125I-labeled TSP was added to each dish, and cells were incubated for an additional 1 or 4 h at 37 °C, still in the presence of enzymes. The cell layers were then washed as described above, and the amount of TSP bound to the cell layer was determined by counting radioactivity solubilized in sodium hydroxide. Degradation of 125I-TSP by enzyme-treated cells was assayed at 1 and 4 h by determination of trichloroacetic acid-soluble radioactivity in the binding medium, as described above. Control cell layers were incubated as described, but in the absence of enzymes. Results are expressed as mean of duplicate samples. The concentrations of enzymes used in these assays were the minimal concentrations that maximally decreased binding, as determined in dose-response assays. In parallel experiments, the enzyme preparations were found to have no proteolytic activity against "1-TSP as determined by trichloroacetic acid precipitation or polyacrylamide gel electrophoresis followed by autoradiography.

Immunofluorescence Localization of TSP Binding to CHO Cells—For indirect immunofluorescence localization of bound TSP, CHO wild type and mutant cells grown to near confluency on glass coverslips were washed three times with 0.01 M phosphate, 0.15 M sodium chloride, pH 7.4 (PBS), and once with F-12/BSA. Coverslips were then incubated with 5 or 50 μg/ml human TSP in F-12/BSA or with F-12/BSA alone for 45 min at 37 °C. Coverslips were washed three times with PBS and fixed for 20 min with 3.5% paraformaldehyde in 0.1 M sodium phosphate, pH 7.4. After three further washes in PBS, coverslips were incubated for 30 min at room temperature with the IgG fraction of a mouse monoclonal antibody to human thrombospondin in PBS containing 0.1% BSA (10). This antibody did not recognize endogenous TSP synthesized by CHO cells and therefore could only label the exogenous TSP. Wild type and mutant 803 and 677 cells were incubated with 25 μg/ml IgG, and the 745 mutant cells were incubated with 125 μg/ml IgG. Coverslips were then washed three times with PBS and incubated with a 1:100 dilution of fluorescein (fluorescein isothiocyanate)-conjugated goat anti-mouse IgG (Jackson Labs, Avondale, PA) in PBS containing 0.1% BSA. Coverslips were washed three times with PBS, mounted on glass slides with glycerol gel (Sigma), viewed by epifluorescence and phase illumination in a Nikon Optiphot microscope, and photographed with a FX-II camera attachment.

Analysis of Sulphatides—The amount of sulfatide was estimated after labeling approximately 106 cells with 20 μCi/ml of [35S]SO4 for three days. After removing the medium, the cell layer was solubilized at room temperature in 0.1 N ammonium hydroxide. Chloroform and methanol were added to achieve a mixture of chloroform/methanol/water (2/3/1, v/v) and 50 μg of beef brain sulfatide (Sigma) was added as carrier. The extract was passed over a small column of silicic acid in chloroform/methanol/water to remove the cholesterol contaminants, and the column was washed with solvent. The effluent was evaporated under a stream of nitrogen and analyzed by thin-layer chromatography on silica gel 60 (Merck, Darmstadt, Federal Republic of Germany) in chloroform/methanol/acidic acid (65/25/10, v/v). The position of carrier sulfatide was determined by exposure of the plate to iodine vapor and to anthrone reagent. The plate was scraped, and the sulfatide content was measured by liquid scintillation counting in a Beckman LS 1800 system. For each cell line, approximately 70% of the radioactive lipids co-chromatographed with the carrier; the remainder was in two other spots that each contained about 15% of the remaining radioactivity. The amount of sulfatide in the major spot was calculated using previously determined specific activity of [35S]SO4 (34).

RESULTS

Binding and Degradation of TSP by Wild Type CHO Cells—Wild type CHO cells bound and degraded TSP via a saturable mechanism similar to that demonstrated for endothelial cells and fibroblasts (27, 28). Studies of the kinetics of binding of labeled TSP to wild type cells showed that binding to cell monolayers rapidly increased during the first 60 min, leveled off at 90–120 min, and was stable for at least 4 h (Fig. 1A). The rates of binding and maximum amounts of TSP bound were comparable in assays performed at 37 and 4 °C. Based on these assays and experiments with endothelial cells (28), it does not appear that TSP binding sites are down-regulated, although this may occur after longer incubation periods (greater than 4 h) that were not examined. Degradation of TSP was first detectable after 60 min of incubation at 37 °C and progressed linearly for the subsequent 5 h (Fig. 1B). There was no detectable degradation at 4 °C (data not shown).

Binding at 4 °C was reversible (Fig. 2). The extent of loss of TSP from the monolayer was greater in the presence of heparin than in the presence of unlabeled TSP or medium alone. Specific binding of TSP, defined as the difference between the total binding and the binding in the presence of 1 μg/ml heparin, was saturable (Fig. 3A). Nonspecific binding at TSP concentrations less than 25 μg/ml was 5–10% of the total binding. The dissociation constants (Kd) obtained by Scatchard analysis (40) of data obtained at 4 °C was 40 nM. The numbers of binding sites per cell were estimated to be 420,000 at 4 °C. Degradation of TSP at 37 °C was saturable (Fig. 3B). Lineweaver-Burke analysis of data obtained between 1 and 4 h of incubation at 37 °C yielded a Kd of 65 ± 7.0 mean of TSP. Therefore, the lack of degradation at 4 °C is probably not caused by an inability of TSP to bind or by an inability of the binding sites to mediate degradation.

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binding of TSP to wild type cells. Also in contrast to binding and degradation of TSP by wild type cells, binding of TSP to the wild type cells, binding of TSP to the five mutant strains lacking xylosyl-(745) or galactosyl-(761) transferases bound only 10–15% of the amount of TSP bound by the wild type cells when assayed at 5 μg/ml (10 nM) TSP (Table II). The reduced level of binding did not reflect a difference in kinetics, since binding to mutant 745 cells was only slightly above background levels even after 6 h of incubation with labeled TSP (Fig. 1A). Of this reduced amount, <50% was sensitive to inhibition by heparin, as compared to >90% for binding of TSP to wild type cells. Also in contrast to binding to the wild type cells, binding of TSP to the 745 and 761 mutants was not saturable (Fig. 4A). Rather, when mutant monolayers were incubated with increased concentrations of TSP, the percentage of TSP bound increased at concentrations >10 μg/ml (22 nM). Degradation of TSP by the 745 and 761 mutants was also reduced as compared to wild type cells (Fig. 1B and Table II), and degradation was relatively insensitive to inhibition by heparin (Fig. 4B). Degradation of labeled TSP by the 745 and 761 mutants was not saturable (Fig. 4B).

Strains specifically defective in heparan sulfate but with normal (803) or elevated (677) chondroitin sulfate also had reduced levels of binding and degradation of TSP as compared to wild type when labeled TSP was tested at 5 μg/ml (Table II). The proportions of total binding and degradation inhibitable by heparin were 30 and 61% for the 803 cells and 51
Proteoglycan-mediated Metabolism of TSP

TABLE I

CHO cell mutants defective in glycosaminoglycan (GAG) biosynthesis

<table>
<thead>
<tr>
<th>Cell</th>
<th>Mutation</th>
<th>Phenotype</th>
<th>Sulfatide content</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>Wild type</td>
<td>Normal GAGs</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>605</td>
<td>Defect in SO₄ transport</td>
<td>Normal GAGs</td>
<td>Not tested</td>
</tr>
<tr>
<td>745</td>
<td>Defect in xylosyltransferase</td>
<td>GAG deficient</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>761</td>
<td>Defect in galactosyltransferase I</td>
<td>GAG deficient</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>803</td>
<td>Undetermined</td>
<td>Heparan sulfate deficient</td>
<td>12.7 ± 1.8</td>
</tr>
<tr>
<td>677</td>
<td>Undetermined</td>
<td>Heparan sulfate deficient, excess</td>
<td>0.7 ± 0.0</td>
</tr>
</tbody>
</table>

**Mutant**

- Normal GAGs
- Normal GAGs
- GAG deficient
- GAG deficient
- Heparan sulfate deficient
- Heparan sulfate deficient, excess
- Chondroitin sulfate

**Estimates are of the major spot of radioactivity that co-chromatographed with sulfatide from beef brains (see "Materials and Methods"). The distribution of radioactivity in major and minor spots did not vary among cell strains. Mean ± S.D.**

TABLE II

Comparison of binding and degradation of [125]TSP by CHO mutants

<table>
<thead>
<tr>
<th>Cell</th>
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<th>Degraded*</th>
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<td></td>
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<td>605</td>
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</tr>
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<td>48</td>
<td>23</td>
</tr>
<tr>
<td>677</td>
<td>129</td>
<td>62</td>
</tr>
</tbody>
</table>

**Confluent monolayers of cells were incubated with 5 μg/ml [125]TSP for 2 h at 4 °C, the time at which maximal binding occurred. Cells were washed three times with F-12/BSA, and bound radioactivity was determined after solubilization in 1 N sodium hydroxide. Results are the mean of duplicates from one representative assay.**

**Degradation was monitored by assaying for trichloroacetic acid soluble radioactivity in aliquots of binding media after 6 h of incubation at 37 °C with 5 μg/ml TSP.**

and 86% for the 677 cells. The small amount of binding to 803 cells corresponds to the 15–20% residual amount of heparan sulfate proteoglycan detected in these mutants. There was variability in the data obtained with 677 mutants. However, half of the values obtained from separate experiments were in the range of 50–72% of the wild type. Saturation experiments performed on both cell lines over TSP concentrations of 1–10 μg/ml indicated a small number (53,000–87,000) of high affinity (Kₐ = 3.6–11 nM) heparin-inhibitable binding sites. However, in experiments in which higher concentrations (>20 μg/ml) of TSP were used, there was no evidence of saturability of TSP binding, and the 677 cells bound more TSP than the wild type cells did at equivalent TSP concentrations (Fig. 5A). Degradation of TSP by mutants 803 (data not shown) and 677 was not saturable (Fig. 5B).

Mutant 605 is defective in sulfate transport; however, these cells make fully sulfated glycosaminoglycans using sulfate derived from cysteine and methionine catabolism (34). Mutant 605 bound and degraded TSP like the wild type (Table II). Although the actual amounts of [125]TSP bound or degraded by either the wild type or mutant cells varied between experiments, the relative amounts of binding and degradation between cell types were consistent over months of experiments and various preparations of labeled and unlabeled TSP.

To test if the inhibition of uptake of TSP in the mutants was selective, the uptake of [125]labeled iDL was examined. Mutant 745, which was grossly defective in TSP metabolism, bound LDL in amounts comparable to wild type cells: 104 ng of LDL/mg of cell protein versus 151 ng of LDL/mg of cell protein, respectively. Both cell strains degraded comparable amounts of LDL as well.

**Immunofluorescence Localization of Bound TSP**—Immunofluorescence localization of added human TSP bound to wild type cells after 45-min incubation at 37 °C with 5 μg/ml TSP demonstrated prominent punctate structures over the surface of the cell (Fig. 6A). There was no detectable staining of added TSP in the matrices of these cells and no staining of cells incubated without exogenous TSP (Fig. 6C). Mutant 677 cells stained heterogeneously, with some cells showing intense staining and others no detectable fluorescence (Fig. 6B). Staining of 803 and 745 cells was minimal (Figs. 6, c and d). Wild type and 677 cells incubated with 50 μg/ml TSP had more intense punctate staining, whereas the 803 and 745 cells had diffuse background staining (not shown).

**Sensitivity of TSP Binding and Degradation to Glycosaminoglycan Degrading Enzymes**—Wild type and mutant cells were treated with glycosaminoglycan degrading enzymes to
determine whether binding and degradation of TSP were dependent upon intact glycosaminoglycans. Both binding and degradation of labeled TSP by wild type CHO cells were sensitive to heparitinase treatment of cell monolayers and insensitive to treatment with chondroitinase ABC (Table III). However, wild type cells that were incubated simultaneously with both enzymes demonstrated a greater reduction in the amount of TSP bound or degraded than cells incubated with heparitinase alone (Table III). Neither of the heparan sulfate-deficient mutants (677 and 803) demonstrated any decrease in binding or degradation when treated with heparitinase. The 677 mutant, however, bound and degraded less TSP when cells were exposed to chondroitinase (Table III). Proteases did not appear to be responsible for the reduced binding and degradation observed in cells following heparitinase/chondroitinase treatments, since there was no degradation of enzyme-treated 125I-TSP as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

**Analysis for the Presence of Sulfatides on CHO Cell Membranes**—To test the possibility that the altered binding and degradation of TSP by the mutants was the result of diminished sulfatide content, mutant and wild type CHO cells were labeled for 3 days with 35SO4, and 35S-labeled lipids were analyzed by thin-layer chromatography (Table I). Although there was considerable variability from analysis to analysis, the 745, 761, and 803 mutants reproducibly incorporated more label into sulfatides than the 677 mutants or wild type cells. Since 10^7 cells equals about 1 μg of cell protein, there must exist approximately 4.2 x 10^9 sites/μg of cell protein to account for 4.2 x 10^9 TSP binding sites/cell. Using Avogadro's number, we calculated that 7 fmol of sulfatide/μg of cell protein is required to account for this number of binding sites assuming that binding of TSP to sulfatide is one-to-one. Using these figures, we calculated that the sulfatide on the 761 and 745 mutants could account for approximately 3.4 x 10^5 binding sites.

![Fig. 5. Binding (A) and degradation (B) of TSP by 677 mutant CHO cells with deficient heparan sulfate and extra chondroitin sulfate. Binding and degradation assays were performed as described in Fig. 3: closed circles, total binding or degradation; open circles, nonspecific binding or degradation as defined by binding or degradation in the presence of 1 μg/ml heparin; and open triangles, specific binding or degradation defined as the difference between total and nonspecific binding or degradation. The expanded scale is used to facilitate comparison with Figs. 3 and 4.](image)

![Fig. 6. Immunofluorescence microscopy of cell surface distribution of TSP bound to wild type and mutant CHO cells. Confluent cell layers were incubated for 45 min at 37 °C with either 5 μg/ml TSP in F-12/BSA (a-d) or F-12/BSA alone (e), fixed with paraformaldehyde, and incubated with a mouse monoclonal antibody to TSP followed by a fluorescein-labeled goat anti-mouse IgG. The cells were wild type (a and e), 677 mutant lacking heparan sulfate and having extra chondroitin sulfate (b), 803 mutant specifically deficient in heparan sulfate (c), and 745 mutant deficient in all glycosaminoglycans (d). The 745 cells were incubated with 5-fold more anti-TSP than the other cells. Exposure times were constant. Micrographs were photographed with the microscope focused on the plane of cell-substratum contact. The punctate staining in a and b was present in all focal planes, following the contours of the cell surfaces (magnification × 390).](image)

**Table III**

<table>
<thead>
<tr>
<th>Binding</th>
<th>Control</th>
<th>Heparitinase</th>
<th>Chondroitinase</th>
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<table>
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**DISCUSSION**

These studies provide evidence that cell surface proteoglycans are required for the receptor-mediated binding and degradation of TSP by CHO cells. Mutant 745 and 761 cells, which are defective in enzymes required for initiation of glycosaminoglycan chain biosynthesis and globally deficient in glycosaminoglycans, bound and degraded much less TSP than the wild type cells when incubated with low concentrations of TSP. Although considerable amounts of TSP bound to the glycosaminoglycan-deficient mutants at higher TSP concentrations, this binding (a) was not saturable, (b) was not accompanied by degradation of TSP, and (c) did not result in the characteristic punctate pattern of immunofluorescence staining.

Additional data suggest that heparan sulfate is the primary cell surface mediator of TSP metabolism. First, the 803 mutant with deficient heparan sulfate and essentially normal chondroitin sulfate was grossly deficient in TSP binding and degradation. Second, binding and degradation of TSP by wild type cells were reduced 2- to 3-fold by treatment with heparitinase whereas chondroitinase had no effect. Nevertheless, chondroitin sulfate seems to serve as a secondary mediator of TSP binding and degradation. Treatment of the wild type cells simultaneously with heparitinase and chondroitinase reduced binding and degradation beyond the reduction found with heparitinase alone. One could hypothesize that chondroitinase digestion of chondroitin sulfate chains facilitated a more complete digestion of heparan sulfate chains by increased accessibility of heparan sulfate to otherwise masked heparan sulfate chains. Such a hypothesis, however, cannot account for the specific, albeit variable, binding and degradation of TSP by the 677 mutants that lack heparan sulfate and have extra chondroitin sulfate. The extra chondroitin sulfate proteoglycan present in the mutants may be of sufficient density on the cell surface to mediate TSP binding. Chondroitin sulfate chains do not appear to be effective mediators of TSP degradation, since when tested at 100 μg of input TSP, only 10% of the TSP bound to the 677 mutants, and 2% of that bound to the 803 mutants was degraded during a 60-min period, whereas 42% of TSP bound to wild type cells was degraded.

It is not known if the large nonsaturable component of binding of TSP to mutant cells represents a second binding mode that could be saturated if higher TSP concentrations were studied (26) or simply the property of TSP to self-aggregate (27, 41). Immunofluorescence examination of the TSP bound at higher concentrations revealed only diffuse background staining, suggesting that the binding is artifactual and nonphysiological. The possibility that sulfatides mediate the elevated levels of TSP binding to the mutants at high TSP concentrations cannot be excluded, although the amount of sulfatide present on the 761 mutants could only account for 10% of the binding of TSP to these mutants at 100 μg of input TSP. Furthermore, there is no correlation between the amount of sulfatide present and the extent of TSP binding and degradation. Alternately, TSP binding to the mutants may be mediated by the OKM5 antigen, although it is not known if CHO cells have the antigen. However, it appears unlikely that the OKM5 antigen mediates binding and degradation by CHO cells, since binding of TSP was augmented by heparin in these reports (26, 42), whereas binding of TSP to CHO cells was either not affected or inhibited in the presence of heparin.

Heparin has been shown to inhibit binding of other molecules, such as LDL, to cell surface receptors other than proteoglycans (43), and proteoglycans have been suggested to function as receptors for other molecules, including platelet factor 4 (32), antithrombin III (29), collagen (30), and lipoprotein lipase (31). The interaction of TSP with heparan sulfate proteoglycan is of special interest, because the interaction apparently results in receptor-mediated endocytosis. Although the possibility must be considered that the proteoglycan "prevents" or "passes on" TSP to a second molecule that "carries" TSP into the cell, it seems simplest to propose that endocytosis is mediated solely by the proteoglycan. The interaction of TSP with heparin is strong at physiological salt concentration and maintained under mild acid conditions (pH 3–5). If the interaction of TSP with heparan sulfate is similar, the proteoglycan would be expected to remain bound to TSP as TSP transverses endocytic vesicles and enters the lysosome. In other words, TSP released from platelets or synthesized endogenously or by a neighboring cell could bind to a cell surface proteoglycan and signal the internalization of both receptor and ligand from the cell surface. This interaction may be regulated at the ligand level, rather than by the receptor, since the synthesis and secretion of TSP appear to be highly regulated, whereas data from these and previous studies suggest that the TSP binding site is not down-regulated.

Membrane-intercalated heparan sulfate proteoglycans appear to be involved in linking the cytoskeleton to the extracellular matrix, especially in the region of focal adhesion plaques (44). It is interesting to speculate that TSP destabilizes such cell-substratum contacts by removal of the proteoglycan from the cell surface and thus is a "permissive" factor that readies a cell for division or migration. The mutant CHO cells should serve as an informative culture system to test this and other speculations and also to study the cellular metabolism of other glycosaminoglycan-binding proteins.

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Proteoglycan-mediated Metabolism of TSP


