Endocytosis of Different Members of the Small Chondroitin/Dermatan Sulfate Proteoglycan Family*

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The family of small interstitial chondroitin/dermatan sulfate proteoglycans consists of at least three different molecular species: biglycan (proteoglycan I), decorin (proteoglycan II), and proteoglycan-100, which has a glycosylated core protein of about 100 kDa. The core protein of decorin has been shown to be responsible for receptor-mediated endocytosis of this proteoglycan species by a variety of mesenchymal cells. It is now demonstrated that skin fibroblasts and articular chondrocytes endocytose biglycan with an efficiency similar to that of decorin. Uptake of biglycan is also mediated by its core protein and can be inhibited by decorin in a partially competitive manner. In human fibroblasts, endosomal proteins of 51 and 26 kDa, which are known to bind decorin core protein, also interact with biglycan. This interaction can be inhibited by decorin. Bovine articular chondrocytes contained binding proteins of 48 and 25 kDa. Proteoglycan-100 can be distinguished from biglycan and decorin by its low clearance rate, which however, exceeds the rate of fluid phase endocytosis.

Small interstitial chondroitin/dermatan sulfate proteoglycans comprise a subset of the virtually ubiquitous proteoglycans in extracellular matrices. Two members of this family have been defined in terms of the amino acid sequence of their core proteins (1–4). They show extensive homologies and contain a characteristic leucine-rich repeat motif. In most cases, small proteoglycan I bears two glycosaminoglycan chains, hence, its name biglycan (1). Small proteoglycan II, which contains only a single glycosaminoglycan chain, has been named decorin because it decorates type I collagen fibrils (5–7). Recently, a third member of the small proteoglycan family has been described. Its core protein of 78 kDa is linked with an asparagine-bound oligosaccharide, with O-glycosidically linked oligosaccharides and probably with only a single chondroitin sulfate chain of 29 kDa (8). Due to the size of its glycosylated core protein, it has tentatively been named PG-100.

In developing human tissues, the expression and localization of biglycan and decorin were shown to be substantially divergent and sometimes mutually exclusive (9). Age-related changes in the relative proportions of these proteoglycans were found at least in cartilage (10). These findings suggest that each member of the small proteoglycan family fulfills specific functions that, however, are not yet known precisely. One of the biological consequences of the interaction of decorin and fibrillar collagens may concern the rate of fibril formation and the final fibril size (11, 12). No such function has presently been ascribed to biglycan (9). However, both proteoglycans were considered to interact with fibronectin, thereby inhibiting the adhesion of fibroblasts to a fibronectin substrate (13–15). Another important observation concerned the inhibitory effect of decorin on cell proliferation (16), which was proposed to be caused by the formation of inactive complexes between the core protein and transforming growth factor-β (17). Hence, the regulation of the concentration of the small proteoglycans by their rates of biosynthesis and degradation appears to be of great physiological importance.

Previous studies have shown that the biosynthesis of biglycan, decorin, and PG-100 is differentially regulated (18, 19). Comparative studies on the catabolism of the three small proteoglycans, however, are not available in the literature. We have shown previously that, in fibroblast cultures, exogenously supplied decorin is internalized by receptor-mediated endocytosis and subsequently degraded in lysosomes (20, 21). Endogenously synthesized decorin undergoes a secretion-recapture pathway that is influenced by extracellular decorin-binding proteins (22). Endocytosis is probably mediated by 51- and 26-kDa receptor proteins that interact with the protein moiety of this proteoglycan (23). The possible existence of receptor proteins for biglycan and PG-100 has not yet been investigated. In addition, it is important to find out whether different cell types are able to endocytose small proteoglycans for assessing anatomical site and regulation of proteoglycan catabolism. In this paper, we demonstrate that cultured skin fibroblasts and articular chondrocytes are capable of receptor-mediated endocytosis of biglycan and decorin, probably by employing the same binding proteins. PG-100, on the other hand, is internalized much less efficiently.

EXPERIMENTAL PROCEDURES

Materials—The following materials were purchased from the suppliers indicated: collagenase, type B, from Clostridium histolyticum (Boehringer, Mannheim); Pronase from Streptomyces griseus (Calbiochem); nylon membranes, 50- and 90-μm (Schweizerische Seidenzahnbrik, Zurich), alginic acid, medium viscosity (Sigma, A2033); [35S]sulfate (carrier-free) and [35S]methionine (30 TBq/mmol, Amerham-Buchler, Braunschweig). Monospecific antisera against the core proteins of decorin (24) and PG-100 (8) were those used previously. A peptide corresponding to residues 11–14 of the secreted form of biglycan (1) was synthesized by using Fmoc (N-(9-fluorenyl) methylcarbonyl)-protected, PyBOP (benzotriazol-1-yloxytrispyrroli-
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Dinophosphonium hexafluorophosphate-activated amino acids (25) and an automatic peptide synthesizer (Milligen 9050, Eschborn, Germany). The peptide was conjugated with bovine serum albumin and used to immunize a rabbit under analogous conditions as described (8). After several washing steps, they were suspended in 4 ml of Dulbecco's medium containing 2.2 mM CaCl2 and 4% (v/v) fetal calf serum (Conco, Wiesbaden) that had been dialyzed against 0.15 M NaCl. Incubations with [35S]methionine were performed as described previously (8). After 3 more min, the CaCl2 solution was removed, and the beads were further processed as described (28). After several washing steps, they were suspended in 4 ml of Dulbecco's medium containing 5% of fetal calf serum and antibiotics. The medium was changed every second day, and the cells were used for endocytosis experiments after 4-7 days.

Preparation of Proteoglycans—MG-63 osteosarcoma cells that almost had reached confluence in 75 cm2 plastic flasks (Greiner, Nürtlingen) were incubated for 72 h in 25 ml of sulfite-free Eagle's minimum essential medium containing 1.48 MBq/ml of [35S]sulfate and 4% (v/v) fetal calf serum (Conco, Wiesbaden) that had been dialyzed against 0.15 M NaCl. Incubations with [35S]methionine were performed as described previously (8). The medium was then made 70% saturated with (NH4)2SO4. After centrifugation (59,000 g for 20 min), the sediment was dissolved in 2.2 ml culture flask of 0.1 M Tris/HCl buffer, pH 7.4, containing 1.0 NaCl and protease inhibitors (24) and centrifuged again. The supernatant was treated with 20 mg/ml of purified heparan sulfate proteoglycan from bovine aortic proteoglycan (Conco, Wiesbaden) that had been dialyzed against 0.15 M NaCl. Incubations with [35S]methionine were performed as described previously (8). After the third washing step, the 90% complete supernatant was diluted with 20 ml Tris/HCl buffer, pH 7.4, and protease inhibitors to reduce the NaCl concentration of the immune precipitation buffer from 1.0 to 0.15 M. To remove the majority (about 90%) of secreted heparan sulfate proteoglycans, the solution was applied, in portions, to a Bio-Gel TSK DEAE-5PW column (7.5 mm x 75 mm, Bio-Rad, Munich) operated exactly as described (23). Chondroitin/dermatan sulfate proteoglycans were eluted at the step from 0.35 to 0.58 M NaCl (1 ml). This fraction was mixed with an equal volume of 0.1 M sodium acetate, pH 5.8, containing 8 g guanidinium chloride and protease inhibitors and dialyzed extensively against serum-free culture medium containing 10 mM HEPES.

The availability of an antisera against a biglycan-decayed peptide made it possible to purify biglycan also by immune precipitation. However, using 90 mg of protein A-Sepharose/culture flask in an incomplete digestion, the tissue fragments were spun down and redigested for 4 h at 37 °C with 5 mg/ml of collagenase.

The chondrocytes obtained were cultivated in alginate beads as described (28). 9 × 10^6 cells were suspended in 225 ml of Dulbecco's medium and 150 ml of 2-fold concentrated Dulbecco's medium, and 1.5 ml of 15% (w/v) algic acid. The cell suspension was dropped between different experiments might be due, at least in part, to the requirement of native proteoglycan core protein structures for endocytosis.

In a separate experiment, proteoglycans that had been digested

RESULTS AND DISCUSSION

Endocytosis of Different Proteoglycans by Monolayer Fibroblasts—Different members of the family of small proteoglycans were isolated from the medium of osteosarcoma cells after labeling with [35S]sulfate (Fig. 1, upper panel). Polycrystalline gel electrophoresis in the presence of sodium dodecyl sulfate showed the expected electrophoretic mobility for all three proteoglycans under investigation (2, 8, 24, 32). Biglycan showed similar electrophoretic mobilities, regardless of whether the proteoglycan had been obtained by specific immune precipitation or by chromatographic procedures. In a separate experiment, proteoglycans that had been digested

Endocytosis—Endocytosis of proteoglycans by skin fibroblasts in monolayer culture was determined as described (21). Cells were plated in 35-mm plastic dishes 1-2 days before the experiment. Labeled proteoglycans were added in a total volume of 1 ml. A pH of 7.4 was dialyzed against peptidase and trypsin protease inhibitors and immediately chromatographed on a 5-ml column of DEAE-Trisacryl (Serva, Heidelberg) as described (24). Peak fractions, which were obtained after elution with 1 M NaCl in 20 mM Tris/HCl, pH 7.4, were mixed with fetal calf serum and dialyzed against 2.2 mM CaCl2 and 4% (v/v) fetal calf serum, that was cleared from the proteoglycan/h and mg of cell protein. Degradation was defined as the sum of the intra- and extracellular amounts of ethanol-soluble radioactivity over the total amount of endocytosed material. The results of duplicate incubations within an experimental series differed by less than 10%. The phenomena to be described were reproducibly observed, although the absolute values for clearance rates or radioactivity of [35S]labeled proteoglycans varied up to a factor of 2 in separate experiments. This high degree of variability between different experiments might be due, at least in part, to the requirement of native proteoglycan core protein structures for endocytosis.

In the case of cultures maintained in an alginate gel, 1.6 g of cell-containing gel beads were mixed with 5 ml of endocytosis medium, distributed over a surface of 25 cm2, and incubated for 22 h at 37 °C. At the end of the incubation, medium was carefully removed, and the gel particles were washed three times with Hank's balanced salt solution and then dissolved by incubation for 10 min at 37 °C with 25 mM sodium hydroxide. The cell suspension was treated with trypsin, and the further processing was performed as described for monolayer cultures. The amount of ethanol-soluble radioactivity was determined in the citrate solution, too. Endocytosis experiments in suspension culture were performed in 35-mm bacteriological plastic dishes 18 h after releasing the cells from alginate beads.
MG-63 osteosarcoma cells are known to produce biglycan, decorin, and PG-100 (8). For a comparative study on their uptake properties, $[^{35}S]$sulfate-labeled proteoglycans were therefore prepared from radioactive secretions of these cells by sequential immune precipitation and chromatographic procedures. Under the assumption that a single pool of 3'-phosphoadenylyl $[^{35}S]$sulfate is used for the biosynthesis of these proteoglycans, they contain sulfate ester groups of identical specific radioactivity. Taking into account the differences in glycosaminoglycan chain length and number (8), molar endocytosis rates can be calculated. It is shown in Table I that fibroblasts endocytosed decorin and biglycan with similar efficiency. When expressed as the volume of medium cleared from the radioactive ligand per unit time and cell protein, the involvement of a receptor-mediated uptake process becomes evident because fluid phase uptake can account for maximally 0.05 $\mu l \times h^{-1} \times mg$ of cell protein$^{-1}$ in fibroblasts (34). The clearance rates obtained for PG-100 were about 2 $\mu l \times h^{-1} \times mg$ of cell protein$^{-1}$. These rates are 1 order of magnitude lower than those for decorin and biglycan but clearly higher than expected for bulk uptake. All three proteoglycans were degraded with similar efficiency, as indicated by the formation of inorganic $[^{35}S]$sulfate.

Endocytosis of biglycan was linear with time (Fig. 2). As could be expected from the necessity of transport to the lysosomal compartment for degradation (35), liberation of inorganic $[^{35}S]$sulfate was delayed.

In spite of the evidence for receptor-mediated endocytosis of biglycan, saturation kinetics were not observed when fibroblasts

with chondroitin ABC lyase were subjected to SDS-PAGE and Western blotting followed by staining with monoclonal and polyclonal antibodies. The results indicated the specificity of the polyclonal anti-biglycan and anti-decorin antisera (Fig. 1, lower panel). Since the antisera against decorin was raised against the chondroitin ABC lyase-digested proteoglycan, the antisera reacted also with the glycosaminoglycan stubs produced by the enzyme (33). Consequently, biglycan also gave a positive immune reaction with polyclonal anti-decorin antibody upon chondroitin ABC lyase treatment. Biglycan was not precipitated, however, by the polyclonal anti-decorin antisera in an undigested state (Fig. 1, upper panel). $[^{35}S]$Methionine-labeled biglycan yielded exclusively the 50-kDa core protein band after chondroitin ABC lyase digestion (result not shown).

![Fig. 1. Electrophoretic and immunological characterization of proteoglycan preparations. Upper panel, fluorograms of $[^{35}S]$sulfate-labeled proteoglycans. Proteoglycans were obtained from the medium of MG-63 cells by immune precipitation of either decorin (D) or biglycan (B). Immune precipitation of decorin was followed by immune precipitation of PG-100 (left part). In the right part, biglycan was purified either by specific immune precipitation (IP) or by conventional chromatographic procedures (CC). The samples were separated on a 4-12.5% acrylamide gradient gel in the presence of sodium dodecyl sulfate. The migration distance of $M_1$ standards (given as $10^{-1} \times M_1$) is shown in the right margin. Lower panel, Western blots of decorin (D) and biglycan (B) core proteins. Biglycan was immune-precipitated from 100 ml of conditioned media from MG-63 cells and processed as described under "Experimental Procedures" for SDS-PAGE (acrylamide gel concentration, 12.5%) and Western blotting. About 0.8 pg of decorin core protein from fibroblast secretions were applied per lane for control purposes. Monoclonal antibodies (mAb) against decorin and polyclonal antisera (pAb) against biglycan and decorin, respectively, were used as the primary immune reagent. Note that the antisera against biglycan may react also with bovine serum albumin (66 kDa), since an albumin-conjugated biglycan peptide was used as antigen.

![Fig. 2. Time dependence of endocytosis (O) and intracellular degradation (A) of biglycan. Biglycan (1 nmol/ml) was added to fibroblasts (110-125 $\mu g$ of cell protein/plate) in monolayer culture. The amounts of endocytosed and degraded biglycan were determined at the times indicated. Results were normalized to a mean protein content of 120 $\mu g$/plate.

![Table I. Uptake, clearance, and degradation rates of Proteoglycans by fibroblasts in monolayer culture. Skin fibroblasts (0.20-0.24 mg of cell protein) were challenged for 6 h with $[^{35}S]$sulfate-labeled proteoglycans from the secretions of osteosarcoma cells. The specific radioactivity was 5,000 cpm/nmol of hexuronic acid, yielding mean specific radioactivities of 9.4 Bq/pmol for decorin, of 16.4 Bq/pmol for biglycan, and of 5.9 Bq/pmol for PG-100, respectively.

<table>
<thead>
<tr>
<th>Proteoglycan</th>
<th>Concentration</th>
<th>Uptake</th>
<th>Clearance</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/ml</td>
<td>pmol/dish</td>
<td>$\mu l \times h^{-1} \times mg^{-1}$</td>
<td>% of endocytosed amount</td>
</tr>
<tr>
<td>Decorin</td>
<td>125</td>
<td>4.2</td>
<td>28</td>
<td>48</td>
</tr>
<tr>
<td>Biglycan</td>
<td>93</td>
<td>4.1</td>
<td>37</td>
<td>61</td>
</tr>
<tr>
<td>PG-100</td>
<td>225</td>
<td>0.65</td>
<td>2.4</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>443</td>
<td>1.1</td>
<td>2.1</td>
<td>59</td>
</tr>
</tbody>
</table>
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Blacks were incubated with up to 3.7 nmol/ml of biglycan (Fig. 3). Half-maximal uptake of decorin had previously been shown to occur at a concentration of about 1 nmol/ml (20). This discrepancy could be explained by a lower affinity of the putative endocytosis receptor(s) for biglycan or by a concentration-dependent self-association of biglycan. Self-association of biglycan but not of decorin has been described (32).

In the experiments described, biglycan obtained by selective removal of other proteoglycan species was used and, hence, could be contaminated with small amounts (less than 5%) of these proteoglycans. Therefore, biglycan purified by immune precipitation was also used as radioactive ligand. In this experiment, proteoglycan containing 5,000 to 50,000 cpm of radioactive label were added per plate. Clearance rates between 66 and 86 μl × h⁻¹ × mg of cell protein⁻¹ were measured, and between 18 and 24% of the added dose became internalized within the experimental period of 24 h. These high uptake rates can only be explained by receptor-mediated endocytosis of biglycan itself.

Previous studies had shown that the glycosaminoglycan-free decorin core protein was the ligand for its endocytosis receptor (21). Analogously, the uptake of [³⁵S]methionine-labeled intact and of glycosaminoglycan-free biglycan was compared (Table II). The results indicated that the core protein alone was sufficient for efficient uptake and that the polysaccharide chains were not only unnecessary but even inhibitory.

Inhibition of Biglycan Uptake—Since biglycan and decorin have highly homologous core protein sequences (1–4), the influence of decorin on the endocytosis of biglycan was tested. It is shown in Fig. 4 that in the presence of increasing doses of decorin, the endocytosis of biglycan became progressively inhibited. When the data were analyzed according to Dixon (36), i.e. the reciprocal value of endocytosis was plotted versus the dose of the inhibitor, nonlinear curves were obtained. Such curves are proposed to be characteristic for a partially competitive inhibition mechanism.

Endocytosis of Small Proteoglycans by Chondrocytes—Articular cartilage has been shown to contain biglycan, as well as decorin (37). Due to the possible involvement of small proteoglycans in the pathogenesis of osteoarthritis, it was of interest to investigate the capability of articular chondrocytes to endocytose these matrix molecules. To avoid the risk of dedifferentiation of chondrocytes in monolayer culture, chondrocytes and, for comparison, fibroblasts were cultured in alginate gels where the chondrocytic phenotype can be maintained for several weeks (28). It is shown in Fig. 5 that both cell types internalized biglycan as well as decorin, although chondrocytes exhibited lower uptake rates for both proteoglycans. It should be stressed, however, that a quantitative interpretation of the data is difficult, because compared to decorin, smaller amounts of biglycan diffused into the alginate gel, and the amount of proteoglycan within the gel was also dependent on the cell type used. Nevertheless, these shortcomings should not detract from the main conclusion that chondrocytes are capable of endocytosing biglycan and decorin in a manner also suggestive of receptor-mediated uptake. In a separate experiment, 10⁷ chondrocytes (580 μg of cell protein) in suspension culture were challenged with either 2.2 × 10⁸ cpm [³⁵S]sulfate-labeled biglycan (●) or 0.2 × 10⁸ cpm (400 pmol) of decorin for 26 h in a total volume of 2 ml. 16.0 and 16.1 pmol of biglycan and decorin, respectively, were endocytosed, corresponding to clearance rates of 3.2 and 5.3 μl/h and mg of cell protein for these two proteoglycans. These data do not allow a comparison with the uptake properties of fibroblasts in monolayer cultures but support the conclusions drawn from the experiments with gel cultured cells.

Biglycan-binding Proteins in Fibroblasts—We had previously shown that decorin binds to proteins of 51 and 26 kDa that are enriched in endosomes and were therefore considered as receptor proteins (23). Several additional proteins of about 30 and 14 kDa, respectively, also exhibited decorin-binding properties. These proteins, however, were not sensitive to trypsin treatment of intact cells and, hence, did not appear to be present at the plasma membrane. A great proportion of these proteins was removed during endosome preparation (23). When extracts of whole fibroblasts were subjected to SDS-PAGE and Western blotting, biglycan could be shown to bind to the 51- and 26-kDa proteins and to the other proteins binding decorin (Fig. 6). In addition, several biglycan-binding proteins with molecular weights around 80,000 were

**Table II**

Endocytosis of biglycan core protein by fibroblasts in monolayer culture

Skim fibroblasts (0.12–0.14 mg of cell protein) were incubated for 6 h with 6,200 cpm of [³⁵S]methionine-labeled biglycan or biglycan core protein. The biglycan used had been isolated by immune precipitation employing an antiserum against a synthetic peptide.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Clearance</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μl × h⁻¹ × mg⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>Intact biglycan</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>Biglycan core protein</td>
<td>152</td>
<td>86</td>
</tr>
</tbody>
</table>
Proteoglycan added (nmoles)

FIG. 5. Endocytosis of biglycan (A) and decorin (B) by fibroblasts (●) and chondrocytes (▲) maintained in an alginate gel. Cells (about 290 μg of cell protein in the case of fibroblasts and about 850 μg of protein in the case of chondrocytes) were entrapped in a gel volume of 1.6 ml. After 2 days (fibroblasts) or 5 days (chondrocytes), the cell-containing gels were incubated for 22 h in 25-cm² cell culture flasks in a horizontal position with 5 ml of endocytosis medium containing the labeled proteoglycan. At the end of the incubation period, about 0.3% (fibroblasts) and 1.0% (chondrocytes) of the initially applied biglycan and about 3.3% (fibroblasts) and 3.9% (chondrocytes) of the applied decorin, respectively, were found in the alginate gel.

FIG. 6. Biglycan-binding proteins in cell extracts from human skin fibroblasts. Monolayer fibroblasts were extracted with SDS-PAGE sample buffer and the extract (100 μg/lane) was subsequently subjected to SDS-PAGE and Western blotting. After incubation with [35S] sulfate-labeled biglycan (0.3 nmol/ml) in the absence (lane 1) or presence (lane 2) of unlabeled decorin (5 nmol/ml), bound ligand was visualized by autoradiography. For comparison, decorin-binding proteins in a similar extract are shown in lane 3. The migration distance of Mₐ standards (given as 10⁻² × Mₐ) is shown in the right margin. Arrows indicate the 51- and 26-kDa proteins, respectively.

Biglycan- and decorin-binding proteins in cell extracts and crude endosomal fractions from bovine chondrocytes. A, a cell extract from chondrocytes (150 μg of protein) was separated by SDS-PAGE. After blotting and incubation with [35S] sulfate-labeled biglycan (50,000 cpm/ml; BGN), bound ligand was visualized by autoradiography. B, postmitochondrial supernatants (65 μg of protein/lane) from chondrocytes were subjected to SDS-PAGE and Western blotting. After incubation with [35S] sulfate-labeled biglycan (107,000 cpm/ml; BGN) and decorin (170,000 cpm/ml; DCN), respectively, bound ligand was visualized by autoradiography. The migration distance of Mₐ standards (given as 10⁻² × Mₐ) is shown in the right margin. Arrows indicate the 48- and 25-kDa proteins, respectively.

Biglycan- and Decorin-binding Proteins in Chondrocytes—To investigate the presence of small proteoglycan-binding proteins in bovine chondrocytes that could be candidates for receptor proteins, post-mitochondrial supernatants were analyzed for proteoglycan binding and compared with the binding properties of whole cell extracts. It is shown in Fig. 7 that a greater proportion of proteoglycan-binding proteins of 48 and 25 kDa were found in the post-mitochondrial supernatant than in the whole cell extract. These proteins could be characterized by their interaction with biglycan as well as with decorin. They may represent the homologues of the human 51- and 26-kDa proteins.

CONCLUSIONS

The results described in the present paper demonstrated facilitated uptake of all three proteoglycans under investigation but also showed that biglycan and decorin were endocytosed with greater efficiency than PG-100. Several arguments suggested that biglycan and decorin shared the same uptake mechanism. Decorin was able to inhibit the internalization of biglycan in a partially competitive manner and to quench the interaction of biglycan with binding proteins. On the other hand, saturation of uptake of biglycan was not achieved at doses where saturability of decorin uptake could be demonstrated (20, 21). However, as mentioned above, biglycan is capable of self-association. Considering the type of competition by decorin, it is noteworthy that the putative decorin receptor proteins have binding sites for both decorin core protein and for heparin. Dermatan sulfate also interacts weakly with these proteins, presumably by binding to the heparin-binding sites (38). Complex inhibition kinetics can, therefore, be anticipated when proteoglycans that contain either one or two glycosaminoglycan chains are employed.

Since the present investigation suggests the existence of a common uptake system for biglycan and decorin, it seems unlikely that the extracellular concentrations of both proteo-
glycans could be differentially regulated by the expression of the endocytosis receptors. In the case of decorin, it has been shown that its binding to type I collagen impedes its endocytotic uptake (22). The presence of type I collagen and of other proteoglycan-binding proteins could, therefore, have a profound influence on the catabolism of these small proteoglycans.

The capability of chondrocytes for receptor-mediated endocytosis of biglycan and decorin may explain the finding of excessive intralysosomal storage of glycosaminoglycans in the cells (41). It can now be argued that small proteoglycans contribute substantially to the storage material observed in chondrocytes.

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