Characterization of Heparanase from a Rat Parathyroid Cell Line*

Katarzyna A. Podyma-Inoue‡, Hideyuki Yokote‡, Kazushige Sakaguchi§, Minoru Ikuta¶, and Masaki Yanagishita†

From the ‡Department of Biochemistry, Division of Oral Surgery, and the §Department of Molecular Medicine, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8458, Japan and the ¶Department of Oral Surgery, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8458, Japan

Cell surface heparan sulfate proteoglycans undergo unique intracellular degradation pathways after they are endocytosed from the cell surface. Heparanase, an endo-β-glucuronidase capable of cleaving heparan sulfate, has been demonstrated to contribute to the physiological degradation of heparan sulfate proteoglycans and therefore regulation of their biological functions. A rat parathyroid cell line was found to produce heparanase with an optimal activity at neutral and slightly acidic conditions suggesting that the enzyme participates in heparan sulfate proteoglycan metabolism in extralysosomal compartments. To elucidate the detailed properties of the purified enzyme, the substrate specificity against naturally occurring heparan sulfates and chemically modified heparins was studied. Cleavage sites of rat heparanase were present in heparan sulfate chains obtained from a variety of animal organs, but their occurrence was infrequent (average, 1–2 sites per chain) requiring recognition of both undersulfated and sulfated regions of heparan sulfate. On the other hand intact and chemically modified heparins were not cleaved by heparanase. The carbohydrate structure of the newly generated reducing end region of heparan sulfate cleaved by the enzyme was determined, and it represented relatively undersulfated structures. O-Sulfation of heparan sulfate chains also played important roles in substrate recognition, implying that rat parathyroid heparanase acts near the boundary of highly sulfated and undersulfated domains of heparan sulfate proteoglycans. Further elucidation of the roles of heparanase in normal physiological processes would provide an important tool for analyzing the regulation of heparan sulfate-dependent cell functions.

Heparan sulfate proteoglycans (HSPGs) are widely present in animal cells. They are one of the major constituents of basement membranes and plasma membranes. HSPGs present on the cell surface or in extracellular matrices have an ability to specifically interact with a variety of biologically active molecules including heparin-binding growth factors, cytokines, proteins involved in cell-cell interactions or cell-extracellular matrix interactions, and pathogens, such as viruses, prions, or plasmodia, thereby regulating biological activities of these molecules. Cell surface HSPGs are strategically located to be used for intercepting and regulating biological signals coming into cells. Thus, mechanisms involved in expressing HSPGs with proper carbohydrate modification, in maintaining them on the cell surface, in shedding them from the cell surface, and finally in controlling their endocytosis and intracellular degradation would all play important roles regulating biological functions of HSPGs.

Heparanase, an endo-β-glucuronidase specifically cleaving HS, has drawn much attention for many years for its potential importance in HS metabolism. Heparanase activities have been detected in various tissues and cells, including placenta (3), platelets (4, 5), liver (6), and Chinese hamster ovary cells (7). High levels of heparanase activities also have been attributed to some cancer cells, such as melanoma (8), hepatoma (9) and other carcinomas (10). Although a number of heparanase activities have been studied for the last 20 years, the first human (11–14) and rat (this study; GenBank™ accession number AF184967) heparanases have been cloned only recently. Heparanases related to cancer cells appear to contribute to the disintegration of extracellular matrix and basement membrane by degrading the HSPGs present and therefore facilitating metastasis (11, 12, 14). In addition, heparanases are proposed to release growth factors bound to HSPG either at the cell surface or in the extracellular matrix and enhance cancer growth (15).

Heparanases during the normal cellular processes contribute to physiological degradation of HSPGs (16). Intracellular degradation processes of HS involving heparanases have been found in a variety of cells (17). It has been reported that cell surface HSPGs undergo unique intracellular degradation pathways after they are endocytosed from the cell surface (16). One of the degradation pathways involves a relatively slow and stepwise endoglycosidic degradation of HS by a heparanase, initially generating HS fragments of a specific length (~10 kDa). This degradation process appears to occur within cellular compartments with neutral pH, suggesting the primary localization of heparanase in some extralysosomal compartments (16). HS fragments generated in the first step further undergo another heparanase cleavage in an acidic compartment that generates even shorter HS fragments with an average molecular mass of 5 kDa followed by the final degradation in the lysosome. This stepwise HSPG degradation suggests the presence of functionally distinct HS-degrading compartments, in
which heparanase plays a pivotal role, and potential metabolic processes regulating biological functions of HS.

Detailed enzymatic properties of heparanase have not been fully elucidated because of the limited availability of the enzymatically active protein. Even information on its substrate specificity has been sparse. Enzyme cleavage sites appear to be present on most HS chains but are rather infrequent and consist of a range of structures but not a single type of structure. Thunberg et al. (18) reported susceptibility of a glucuronidic linkage in a defined heparin octasaccharide with anti-thrombin III binding property to a heparanase derived from platelet. Another report partially characterized a minor enzymatic activity among multiple heparanase activities found in Chinese hamster ovary cells, but the major heparanase activity in the system remained elusive (19). In the present study, using a heparanase derived from a rat parathyroid cell line, we have determined a range of heparanase substrate structures found in naturally occurring HS chains with structural diversity. The present study has provided information on the major substrate structure of heparanase and the occurrence of HS chains susceptible to the enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—Centricon 30 ultrafiltration membrane units were purchased from Millipore (Bedford, MA). [35S]Sulfate (250–1000 mCi/mmol) was obtained from PerkinElmer Life Sciences. Trypsin (tosyl-phenylalanyl chloromethyl ketone-treated) was obtained from Sigma. Heparanase (Phascolarctus hemprichii), heparitinase (P. heparinum), chondroitinase ABC (Proteus vulgaris), chondroitin 4-sulfate (whale cartilage), heparin disulfate (shark cartilage), and chemically modified heparins (completely desulfated and N-acetylated heparin, completely desulfated and N-sulfated heparin, and N-desulfated and N-acetylated heparin) were obtained from Seikagaku Corp. (Tokyo, Japan). Chondroitin-4-sulfates and chondroitin-6-sulfates were obtained from bovine kidney, intestine, lung, and aorta were a kind gift from Dr. K. Yoshida of Seikagaku Corp., and their characteristics have been reported previously (20). Total RNA isolation reagent (RNA STAT-60™) was purchased from TEL-TEST, Inc. (Friendswood, TX). Other reagents used were of the highest grades commercially available.

**Preparation of Metabolically Radiolabeled HSPGs—**[35S]-Labeled HSPGs were prepared from metabolically radiolabeled rat osteosarcoma cell (UMR 106) and rat parathyroid (PTr) cells as reported previously (21, 22). Briefly, cell cultures at ~80% confluency were incubated for 16–20 h at 37 °C under 95% air, 5% CO2 in Dulbecco’s modified Eagle’s medium/F/12 (1:1, Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) in the presence of [35S]sulfate at the concentration of 50 μCi/ml. After labeling for 24 h, cells were washed three times with Mg2+- and Ca2+-free phosphate-buffered saline and treated with 10 milliunits/ml chondroitinase ABC for 15 min at 37 °C followed by incubation with trypsin (10 μg/ml) for an additional 10 min. Materials released by trypsin containing cell surface HSPG were collected and centrifuged for 10 min at 3,000 rpm to remove cell debris. The supernatant made up to 4M in guanidine HCl, 0.5% Triton X-100 was applied to a Sephadex G-50 (Amersham Biosciences) column equilibrated with 8 M urea, 0.2 M NaCl, 0.05 M sodium acetate, pH 6.0, containing 0.5% Triton X-100. Reduced HS (250 l) was already dialyzed against 1 M NaBH4, 50 mm NaOH at 45 °C for 24 h and desalted using a Superose Peptide (1 × 30 cm) gel filtration column in 0.65 M NaCl, 50 mM phosphate, pH 7.4 buffer connected to an FPLC system. Heparanase or heparitinase treatment of [3H]-labeled HS was carried out in 0.1 M Tris-acetate buffer, pH 7.4 and 4 °C. The reaction mixture (100 l) was incubated at 37 °C with PTr cell extract as described above. Enzymatic digestion of heparanase from PTr cell extract was analyzed using an FPLC system. Heparinase or heparitinase treatment of [3H]-labeled HS was carried out in 0.1 M Tris-acetate buffer, pH 7.4 and 4 °C. The reaction mixture (100 l) was incubated at 37 °C with PTr cell extract as described above. Enzymatic digestion of heparanase from PTr cell extract was analyzed using an FPLC system.

**RESULTS**

**Detection of Heparanase in Rat Parathyroid Cell Line—**Metabolically radiolabeled HSPG was incubated with PTr cell extract as described under “Experimental Procedures” for different times at 4 °C. A low temperature of 4 °C was used to slow down the enzymatic activity to clarify reaction kinetics. The degradation of HS by intact tissue culture proteases was followed by analysis of smaller fragments (average molecular mass ~10 kDa) which were already visible after 5 min of incubation and increased in a time-dependent manner reaching a plateau by 90 min of incubation (Fig. 1). This process was not inhibited by a mixture of protease inhibitors, suggesting the presence of HS-specific enzyme (data not shown).
Determination of Optimum pH for Enzyme Activity and Inhibitory Effect of Heparin—Most of the heparanase activities reported so far appear to act at acidic pH (14, 27). To determine the optimal degradation conditions for PTr heparanase, the ultrafiltration heparanase assay was carried out at different pH values as described under “Experimental Procedures.” Degradation of 35S-labeled HSPG was extensive at both neutral and slightly acidic pH (Fig. 2). This discrepancy from the previous reports suggested that either the enzyme differs in the biochemical properties from the one reported previously or specific electrolyte compositions at neutral pH used in the present study allowed its optimal activity. Especially it was noted that the presence of divalent cations such as Mg \(^{2+}\) and Ca \(^{2+}\) enhanced the enzyme activity, while the addition of EDTA inactivated it (data not shown). Freeman et al. (28) have reported that heparanases derived from rat liver, B16 melanoma cells, and human umbilical vein endothelial cells as well as rat and human carcinoma cell lines were capable of cleaving heparin, while heparanase from PTr cells did not cleave heparin (Fig. 3A). In fact, heparin strongly inhibited the enzyme activity with an IC\(_{50}\) = 0.048 µg/ml (Fig. 4A). Similarly, all chemically modified heparins tested were not degraded by heparanase (Fig. 3, B and C) but showed variable degrees of enzyme inhibition. Thus, inhibitory activity of intact and chemically modified heparins on the enzyme activity was analyzed to evaluate the relative contribution of specific sulfate groups (Fig. 4B). Analysis using Lineweaver-Burk plots indicated that inhibition of heparanase activity on HS as the substrate by the intact heparin was competitive with \(K_i = 0.032\) µg/ml. The effect of N-desulfation was minimal on the inhibitory activity of heparin (filled triangle) and resulted in a \(K_i\) value of 0.046 µg/ml, while the O-desulfation (filled square) significantly reduced its inhibitory activity of heparin resulting in \(K_i = 1.0\) µg/ml (values represent the average based on multiple experiments). As expected, totally desulfated heparin and hyaluronic acid did not show any inhibitory activity (data not shown). Interestingly both chondroitin 4-sulfate and 6-sulfate showed a significant inhibition with \(K_i = 1.2\) µg/ml (data not shown). Pretreatment of chondroitin sulfate with nitrous acid at pH 1.5 did not alter the \(K_i\) value, indicating that this inhibition was genuine to chondroitin sulfate and not due to contaminating heparin in the chondroitin sulfate preparation.

Analysis of Substrate Structures of Heparanase—HS glycosaminoglycans prepared from bovine lung, kidney, intestine, and aorta were 3H-labeled at their original reducing ends, digested with partially purified heparanase, and analyzed by gel-permeation chromatography on a Superose 6 column.

**Fig. 1.** Time-dependent degradation of 35S-labeled HSPG by rat parathyroid cell extract. Rat parathyroid cell extracts were incubated in the presence of [35S]HSPG on ice for the defined time. Degradation products from different incubation times were analyzed by gel-permeation chromatography on a Superose 6 column.

**Fig. 2.** Optimum pH for rat parathyroid heparanase. The heparanase activity assay was carried out at different pH values as described under “Experimental Procedures.” Data represent relative enzyme activity assuming the highest enzyme activity in Tris buffer, pH 7.0. ▲, acetate buffer; ●, citrate buffer; ◆, Tris buffer.
FIG. 3. Digestion of intact and chemically modified heparins with rat parathyroid heparanase. Intact and chemically modified heparins were labeled at their reducing ends with [3H]borohydride and subjected to heparanase cleavage in the presence of [35S]HSPG as an internal control. A, heparin; B, N-desulfated, N-reacetylated heparin; C, completely desulfated, N-resulfated heparin. Digestion products were analyzed on a Superose 6 column: intact [3H]heparin or chemically modified heparins before digestion ( ), after incubation with PTr heparanase (○), and [35S]HSPG incubated with PTr heparanase as an internal control (△). Profiles of [35S]HSPG were omitted in A and B. In C, an elution profile of intact [35S]HSPG is also shown (▲).

FIG. 4. Inhibition of rat parathyroid heparanase by heparin. Heparanase activity assay was carried out in the presence of heparin (A) as described under "Experimental Procedures." Enzyme activity was expressed as a percentage of untreated control. Incubation with chemically modified heparins (B) confirmed the more important role of O-sulfate groups in the inhibition. ○, control (HS only); ▲, N-desulfated, N-reacetylated heparin; ■, completely desulfated, N-resulfated heparin.
resulted in little change in the chromatogram, indicating that GlcNH$_2$ was not present in the majority of $^3$H-labeled HS fragments (Fig. 6B). Low pH nitrous acid treatment generated extensively degraded HS oligosaccharides (Fig. 6C): $-50\%$ of $^3$H activity in trisaccharide, $8\%$ in pentasaccharide, and the rest in hepta- and larger oligosaccharide positions, indicating that the first GlcNSO$_3$ was present on $50\%$ of HS oligosaccharides on the fourth sugar from the cleavage site, $8\%$ at the sixth sugar, and so on. Heparinase treatment resulted in little digestion, indicating the lack of highly sulfated regions susceptible
to the enzyme on the non-reducing end side near the cleavage site (Fig. 6D). Periodate oxidation generated a peak that contained virtually all the radioactivity at the position corresponding to smaller than monosaccharide size, indicating the absence of 2-O-sulfation on the first GlcUA (Fig. 6F). Heparitinase digested almost all radioactive HS oligosaccharides into two closely eluting products (roughly 50% of radioactivity in each peak) of trisaccharide size (Fig. 6). Analysis of each peak using an ion-exchange column in high performance liquid chromatography suggested that the early eluting peak was a monosulfated trisaccharide, while the later eluting peak was a non-sulfated trisaccharide (data not shown). This suggested that the third sugar from the cleavage site to the direction toward the non-reducing end was GlcUA. Results of the same set of analyses for other HS preparations from other organs were essentially the same except that the proportions of peaks after heparitinase digestion and low pH nitrous acid treatment differed slightly (see Table I). These results were summarized to illustrate the carbohydrate structure of the non-reducing end side of the heparanase cleavage site (Fig. 7). The structural features near the cleavage site were similar among all HS preparations analyzed and represented a range of structures with a relatively undersulfated region of HS chain. The data were consistent with the cleavage with an endo-β-glucuronidase with none of the reducing end GlcUA sulfated. The second residue downstream from the cleavage site was GlcNAc of which ~50% was O-sulfated, thus it was not an obligated sulfation. The third residue was GlcUA. The fourth residue was GlcN of which ~50% was N-sulfated. Since glucuronic linkages between the third and the second and between the fifth and the fourth residue were not susceptible to the heparanase, the GlcN on the reducing end side of the cleavage site may have a different sulfation pattern from those present on the second and fourth GlcN, e.g. containing two or more sulfate residues.

**DISCUSSION**

Most recent studies on heparanase have emphasized its roles in pathological processes such as cancer metastasis (8, 11, 12, 14) or inflammation (29). In the present study, however, we have focused on the function of heparanase in the normal cellular catabolism of HSPG, which is likely to be the main physiological function of the enzyme. The exclusive localization of heparanase in the cell and its absence in the conditioned medium demonstrated in this study indicated its primary function as the enzyme responsible for the intracellular degradation of HSPG as observed in a number of metabolic studies of cell surface HSPGs. Heparanase activities found in blood vessels and tumor masses as reported in other papers (11, 29), on the other hand, were present mostly in the extracellular spaces and therefore appeared to reflect distinct or nonphysiological usages of the enzyme. The optimum pH for the parathyroid heparanase ranged from neutral to slightly acidic, suggesting the enzyme to be functional in prelysosomal compartments in the cell. This was consistent with results from previous experiments demonstrating that endoglycosidic degradation of HSPG in rat parathyroid cells was insensitive to lysosomeotropic agents such as chloroquine (17). Based on these results we postulate that putative localization of rat parathyroid heparanase is in prelysosomal compartments that function at near neutral pH, e.g. endosomes, where HSPGs undergo specific degradation with slow turnover rates (16).

Substrate specificity for the cleavage by heparanase was unique, especially with its infrequent occurrence and the range of carbohydrate structures involved. The presence of infrequent enzyme cleavage sites may be due to either the recognition of a single, unusual modification in HS chains such as GlcNH₂ or sulfated GlcUA by the enzyme or the requirement of extended carbohydrate sequences for the cleavage. Results of the present study indicated that the latter appears to be the case. Involvement of carbohydrate sequences with substantial size is characteristic to protein-carbohydrate binding shared by many heparin- or HS-binding proteins. Despite the presence of heparanase cleavage sites in low frequency, most HS chains with structural diversity prepared from various organs showed susceptibility, except ones from aorta, which may have been already exposed to the enzyme in the tissue.

O-Sulfation of HS seems to be important in both substrate recognition and in the degradation process (Fig. 2A). This was supported by a significant reduction of heparanase-inhibitory activity by O-desulfation of heparin as well as by the inhibition of the enzyme by chondroitin sulfate. On the other hand, lack of highly sulfated residues susceptible to heparinase digestion (Fig. 5D) as well as periodate oxidation (Fig. 5E) on the non-reducing end side of the heparanase cleavage site suggested carbohydrate structures near the reducing end generated upon the cleavage seem to be composed mainly of unsulfated saccharide residues (Fig. 5), implying that rat parathyroid heparanase acts near the boundary of highly sulfated and undersulfated domains of HSPGs. Heparanase cleavage site structure proposed by the present study significantly differs from the one speculated by Bame and colleagues (19). Bame et al. (19) suggested that Chinese hamster ovary heparanase, as well as heparanase derived from placenta and liver, generates two classes of cleavage site structures. The first group (class I), representing the major products, which were not fully characterized, has relatively unmodified structures near the reducing end. It appeared to consist mostly of GlcUA and GlcNAc resi-

---

**TABLE I**

Carbohydrate analysis of heparanase cleavage site

| Source of HS | Percentage of ³H-labeled, non-sulfated trisaccharides after heparitinase digestion | Percentage of ³H-labeled oligosaccharides after low pH nitrous acid treatment |
|-------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Bovine kidney | 44 | Larger than pentasaccharide | 37 |
| 1.25 x fraction | 49 | Pentasaccharide | 14 |
| Bovine intestine | 34 | Trisaccharide (non-sulfated) | 50 (33) |
| Bovine lung | 50 | | |
| Bovine aorta | 30 | | |

*See Ref. 20 for detailed chemical properties of HS preparations.*

---

**FIG. 7. Carbohydrate structure of rat parathyroid heparanase cleavage site.** The structure represents the reducing end generated upon heparanase cleavage (arrow). GlcUA, glucuronic acid; GlcNAc, N-acetylated glucosamine; GlcN₂O₃, N-sulfated glucosamine.

---

**HSPG**

Source of HS | Percentage of ³H-labeled, non-sulfated trisaccharides after heparitinase digestion | Percentage of ³H-labeled oligosaccharides after low pH nitrous acid treatment |
|-------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Bovine kidney | 44 | Larger than pentasaccharide | 37 |
| 1.25 x fraction | 49 | Pentasaccharide | 14 |
| Bovine intestine | 34 | Trisaccharide (non-sulfated) | 50 (33) |
| Bovine lung | 50 | | |
| Bovine aorta | 30 | | |

*See Ref. 20 for detailed chemical properties of HS preparations.*

---

**DISCUSSION**

Most recent studies on heparanase have emphasized its roles in pathological processes such as cancer metastasis (8, 11, 12, 14) or inflammation (29). In the present study, however, we have focused on the function of heparanase in the normal cellular catabolism of HSPG, which is likely to be the main physiological function of the enzyme. The exclusive localization of heparanase in the cell and its absence in the conditioned medium demonstrated in this study indicated its primary function as the enzyme responsible for the intracellular degradation of HSPG as observed in a number of metabolic studies of cell surface HSPGs. Heparanase activities found in blood vessels and tumor masses as reported in other papers (11, 29), on the other hand, were present mostly in the extracellular spaces and therefore appeared to reflect distinct or nonphysiological usages of the enzyme. The optimum pH for the parathyroid heparanase ranged from neutral to slightly acidic, suggesting the enzyme to be functional in prelysosomal compartments in the cell. This was consistent with results from previous experiments demonstrating that endoglycosidic degradation of HSPG in rat parathyroid cells was insensitive to lysosomeotropic agents such as chloroquine (17). Based on these results we postulate that putative localization of rat parathyroid heparanase is in prelysosomal compartments that function at near neutral pH, e.g. endosomes, where HSPGs undergo specific degradation with slow turnover rates (16).

Substrate specificity for the cleavage by heparanase was unique, especially with its infrequent occurrence and the range of carbohydrate structures involved. The presence of infrequent enzyme cleavage sites may be due to either the recognition of a single, unusual modification in HS chains such as GlcNH₂ or sulfated GlcUA by the enzyme or the requirement of extended carbohydrate sequences for the cleavage. Results of the present study indicated that the latter appears to be the case. Involvement of carbohydrate sequences with substantial size is characteristic to protein-carbohydrate binding shared by many heparin- or HS-binding proteins. Despite the presence of heparanase cleavage sites in low frequency, most HS chains with structural diversity prepared from various organs showed susceptibility, except ones from aorta, which may have been already exposed to the enzyme in the tissue.

O-Sulfation of HS seems to be important in both substrate recognition and in the degradation process (Fig. 2A). This was supported by a significant reduction of heparanase-inhibitory activity by O-desulfation of heparin as well as by the inhibition of the enzyme by chondroitin sulfate. On the other hand, lack of highly sulfated residues susceptible to heparinase digestion (Fig. 5D) as well as periodate oxidation (Fig. 5E) on the non-reducing end side of the heparanase cleavage site suggested carbohydrate structures near the reducing end generated upon the cleavage seem to be composed mainly of unsulfated saccharide residues (Fig. 5), implying that rat parathyroid heparanase acts near the boundary of highly sulfated and undersulfated domains of HSPGs. Heparanase cleavage site structure proposed by the present study significantly differs from the one speculated by Bame and colleagues (19). Bame et al. (19) suggested that Chinese hamster ovary heparanase, as well as heparanase derived from placenta and liver, generates two classes of cleavage site structures. The first group (class I), representing the major products, which were not fully characterized, has relatively unmodified structures near the reducing end. It appeared to consist mostly of GlcUA and GlcNAc resi-
dues. On the contrary, the second group (class II), representing the minor product, was suggested to have a structure (GlcNSO$_3$–IdoUA$_2$S–GlcNSO$_3$–HexUA–GlcNAc–GlcUA). The presence of distinct, multiple activities of heparanase could have been due to activities of an enzyme as suggested by authors (19), the action of another enzyme such as a hexosaminidase, or even the presence of multiple heparanases. There is the potential that the class I activity may resemble that of the parathyroid heparanase reported in the present study.

Whether parathyroid heparanase recognizes the structure similar to those proposed by Pikas et al. (9) is still uncertain. Pikas and her colleagues used a heparin octasaccharide with a defined structure that has antithrombin binding property as the substrate and suggested that the reducing end formed upon the heparanase cleavage can be summarized as the following sequence: HexUA–GlcNAc/So$_5$–GlcUA. Although the structure presented by Pikas et al. (9) is compatible with those of our present study, PTr heparanase was found to be totally inhibited by heparin and could not cleave it. Since the only substrate used in the study by Pikas et al. (9) was a short oligosaccharide, the effect of the carbohydrate truncation on the enzyme activity could not be evaluated.

Biological roles of heparanase have been postulated in diverse pathological conditions in addition to their role in cancer metastasis. It is widely recognized that the shedding of HSPG from the endothelium by heparanase causes the loss of the endothelial cell barrier and enables extravasation of blood elements (30). HS fragments generated by heparanase may also stimulate the release of factors responsible for immune cell response. The exact roles of heparanase in normal physiological processes and cell function have been largely unknown. The enzyme participating in the metabolism of cell surface HSPGs is likely to induce the changes in cell functions and structure. The fragments of HS generated during the stepwise degradation by heparanase are likely to possess some biological activities depending on their structure and molecular size. It is possible that HS fragments generated in the stepwise degradation may slow down the degradation of basic fibroblast growth factor and prolong its intracellular life, which consequently could modulate some biological function of basic fibroblast growth factor (31). Tumova et al. (32) showed that a portion of internalized basic fibroblast growth factor was localized to the nucleus together with short HS chains, suggesting that HS fragments may function as a carrier that not only directs fibroblast growth factor to nucleus but also protects it from a rapid degradation. Moreover, HS fragments generated upon heparanase treatment can be released from the cell (29) to the extracellular space where they can function as a competitive inhibitor of HSPGs (33). Further elucidation of the biological roles of heparanase would provide pivotal information on these biological processes.

REFERENCES
1. Bernfield, M., Gottle, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zako, M. (1999) Annu. Rev. Biochem. 68, 729–777
2. Kjellen, L., and Lindahl, U. (1993) Annu. Rev. Biochem. 60, 443–475
3. Klein, U., and Von Figura, K. (1976) Biochem. Biophys. Res. Commun. 73, 569–576
4. Oldberg, A., Heldin, C.-H., Wasteson, Å., Busch, C., and Hook, M. (1980) Biochemistry 19, 5755–5762
5. Oosta, G. M., Favreau, L. V., Beecher, D. L., and Rosenberg, R. R. (1982) J. Biol. Chem. 257, 11249–11255
6. Freeman, C., and Parish, C. R. (1997) Biochem. J. 325, 229–237
7. Bame, K. J. (1993) J. Biol. Chem. 268, 19556–19664
8. Nakajima, M., Murakami, Y., Mishima, Y., Sato, H., and Pecker, I. (1983) Science 220, 611–613
9. Pikas, D. S., Li, J. P., Vlodavsky, I., and Lindahl, U. (1998) J. Biol. Chem. 273, 18770–18777
10. Kosir, M. A., Quinn, C. C., Zakowski, K. L., Grignon, D. J., and Ledbetter S. (1997) J. Surg. Res. 67, 98–105
11. Vlodavsky, I., Friedman, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michel, I., Spector, L., and Pecker, I. (1999) Nat. Med. 5, 793–802
12. Hulett, M. D., Freeman, C., Hamsdorf, B. J., Baker, R. T., Harris, M. J., and Parish, C. R. (1999) Nat. Med. 5, 803–809
13. Kussie, P. H., Hulmes, J. D., Ludwig, D. L., Patel, S., Navarro, E. C., Seddon, A. P., Giorgio, N. A., and Bohlen, P. (1999) Biochem. Biophys. Res. Commun. 261, 183–187
14. Toyoshima, M., and Nakajima, M. (1999) J. Biol. Chem. 274, 24153–24160
15. Whitelock, J. M., Murdoch, A. D., Izzo, R. V., and Underwood, P. A. (1996) J. Biol. Chem. 271, 10079–10086
16. Yanagishita, M., and Hascall, V. C. (1992) J. Biol. Chem. 267, 9451–9454
17. Takeuchi, Y., Yanagishita, M., and Hascall, V. C. (1992) J. Biol. Chem. 267, 14677–14684
18. Thunberg, L., Backström, G., Wasteson, Å., Robinson, H. C., Ogren, S., and Lindahl, U. (1982) J. Biol. Chem. 257, 10278–10293
19. Bame, K. J., and Rohson, K. J. (1997) J. Biol. Chem. 272, 2245–2251
20. Macearana, M., Sakuro, Y., Tawada, A., Yoshiha, K., and Lindahl, U. (1996) J. Biol. Chem. 271, 17804–17810
21. McQuillan, D. J., Findlay, D. M., Hocking, A. M., Yanagishita, M., Midura, R. J., and Hascall, V. C. (1999) Biochem. J. 277, 199–206
22. Sakaguchi, K., Santora, A., Ziming, M., Curcio, F., Aurbach, G. D., and Brandi, M. L. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3269–3273
23. Hascall, V. C., Calabro, A., Midura, R. J., and Yanagishita, M. (1994) in Methods in Enzymology, Vol. 230, pp. 390–417, Academic Press, San Diego, CA
24. Shivley, J. R., and Conrad, H. E. (1976) Biochemistry 15, 3932–3943
25. Yanagishita, M., and Hascall, V. C. (1983) J. Biol. Chem. 258, 12847–12856
26. Yanagishita, M., and Hascall, V. C. (1983) J. Biol. Chem. 258, 12857–12864
27. Freeman, C., and Parish, C. R. (1999) Biochem. J. 330, 1341–1350
28. Freeman, C., Browne, A. M., and Parish, C. R. (1999) Biochem. J. 342, 361–368
29. Dempsey, L. A., Plummer, T. B., Coombes, S. L., and Platt, J. L. (2000) Glycobiology 10, 467–475
30. Platt J. L., Verteletti, G. M., Lindman, B. J., Oegema, T. R., Jr., Bach, F. H., and Dalmasso, A. P. (1990) J. Exp. Med. 171, 1363–1368
31. Burgess, W. H., Shanheen, A. M., Hampton, B., Donohue, P. J., and Winkel, J. A. (1991) J. Cell. Biochem. 45, 131–136
32. Tumova, S., Hatch, B. A., Law, D. J., and Bame, K. J. (1999) Biochem. J. 337, 471–481
33. Ruoslahti, E., and Yamaguchi, Y. (1991) Cell 64, 867–869