Degradation of Endocytosed Dermatan Sulfate Proteoglycan in Human Fibroblasts

Willi Hoppe†, Uwe Rauch, and Hans Kresse

From the Institute of Physiological Chemistry and Pathobiocchemistry, University of Münster, Waldeyerstrasse 15, Münster, Federal Republic of Germany

Endocytosis and subsequent degradation of iduronic acid-rich small dermamn sulfate proteoglycan from fibroblast secretions were studied in human fibroblasts. Upon endocytosis of [3H]leucine- and [35S]sulfate-labeled proteoglycan release of free leucine was 10 to 15 times more rapid than that of inorganic sulfate. Within approximately 3 h a steady state was approached between transport of proteoglycan to the compartment of core protein degradation and release of free leucine. No such steady state could be found with respect to the dermamin sulfate chains. In the presence of benzyloxy carbonyl-Phe-Ala-diazomethylketone or of other SH-protease inhibitors the degradation of the protein moiety of endocytosed proteoglycan was much less inhibited than the degradation of the polysaccharide chain. Benzyloxy carbonyl-Phe-Ala-diazomethylketone did not affect the degradation of dermamin sulfate chains taken up by fluid phase endocytosis and the activities of all known dermamin sulfate-degrading enzymes. Percoll gradient centrifugation indicated that also in the presence of the protease inhibitor the partially degraded proteoglycan accumulated in dense lysosomes. The isolation of intracellular dermamin sulfate peptides and molecular size determinations of endocytosed dermamin sulfate proteoglycan supported the conclusion that a critical proteolytic step is required before the dermamin sulfate chain becomes accessible to hydrolytic enzymes.

Degradation of proteoglycans to their monomeric constituents is an intralysosomal event as concluded from the intralysosomal accumulation of partially degraded glycosaminoglycans in the mucopolysaccharidoses, in which one of the glycosaminoglycan-degrading enzymes has been rendered inactive by mutation (1, 2). Partial proteolytic or endoglycosidic degradation, however, may occur extracellularly (see Ref. 3 for a review) or in a prelysosomal intracellular compartment (4, 5). Different ways of initiating breakdown may be considered. First, the intact proteoglycan is internalized by receptor-mediated endocytosis. This is proposed to be the fate of a ubiquitous small dermamin sulfate proteoglycan (DS-PG)† the protein moiety of which is considered to be recognized by a specific receptor (6-9). Second, a complex of proteoglycan and a second constituent of the extracellular matrix (e.g. fibronectin) which is bound to its membrane receptor is internalized as a result of normal membrane flow. Such a mechanism may operate in the internalization of heparan sulfate proteoglycans (10). Third, the proteoglycan diffuses out of the tissue of origin, maybe after limited proteolysis. It could then be degraded by liver endothelial cells which are equipped with a receptor recognizing hyaluronate and chondroitin sulfate (11, 12).

The transport of proteoglycans from the plasma membrane to the lysosomes has not been studied in detail. Electron microscopic studies showed that after application to cultured smooth muscle cells proteoglycans from arterial tissue were present in coated vesicles, noncoated vesicles, multivesicular bodies, and secondary lysosomes (13). Two intracellular pathways for the degradation of membrane-bound proteoglycans were proposed to operate in rat ovarian granulosa cells (14). One pathway leads to a rapid and complete intralysosomal degradation (t½ of 30 min). In the second more slowly operating pathway (t½ of 4 h) degradation starts with extensive proteolysis and endoglycosidic breakdown in the case of heparan sulfate before final hydrolysis takes place.

Studies on the turnover of proteoglycans are complicated even in the simple model of cultured cells by the fact that cell-associated proteoglycans are localized not only in the different biosynthetic and degradative compartments but also in the nucleus (15) and as integral and peripheral components in the plasma membrane (16). Thus, the disappearance of proteoglycans from the cell comprises exocytosis and shedding as well as intracellular degradation. To get some insight into the interdependence of proteolytic and glycosidic degradation of proteoglycans we have, therefore, used an experimental system in which the degradation of endocytosed iduronic acid-rich small DS-PG could be studied with minimal interference by the other processes of proteoglycan metabolism.

This DS-PG which is the main secretory product of cultured skin fibroblasts (17) is one of the two species of small proteoglycans that carry galactosaminoglycan chains (18). It contains a single dermamin sulfate chain (M, approximately 37,000) which is linked to a serine residue near the N terminus (19). From cloned cDNA a molecular weight of 36,319 for the mature core protein was calculated (20). Additionally, either two or three asparagine-bound oligosaccharides are present (17). It had been shown previously that the core protein of DS-PG is required for receptor-mediated endocytosis, and that lysine and arginine residues are partly responsible for efficient uptake (9).

**EXPERIMENTAL PROCEDURES**

*Materials—Cbz-Phe-Ala-CHN₂, an inhibitor of thiol proteases (21), was kindly donated by Dr. E. Shaw, Friedrich-Miescher-Institut...*
Degradation of Dermatan Sulfate Proteoglycan

5927

tute, Basel. Other materials were purchased from the suppliers indicated: protease inhibitors and cycloheximide from Sigma; chondroitin ABC lyase from Seikagaku Kogyo; sodium [35S]sulfate (specific radioactivity 0.9–1.5 TBq/mg sulfur) and L-[5,5-3H]arginine (specific radioactivity 0.55 TBq/mmol) from Amersham-Buchler; L-[4,5-3H]leucine (specific radioactivity 1.5–2.2 TBq/mmol) from Du Pont–New England Nuclear; DEAE-Trisacryl and Percoll from Pharmacia LKB Biotechnology Inc.

Preparation of Labeled Proteoglycans and Glycosaminoglycans—Skin fibroblasts from healthy juvenile and adult donors were maintained in culture as described (22). For the preparation of labeled proteoglycans cells grown to confluency in 75-cm² Falcon plastic flasks were used. They were incubated with [35S]sulfate in sulfate-depleted and modified Eagle’s minimal essential medium (22) and with [3H]leucine and/or [35S]sulfate in modified leucine-free Waymouth MAB 87/3 medium (17) as quoted earlier. For the preparation of [3H]arginine-labeled proteoglycans the cultures received 1 MBq/ml [3H]arginine in arginine-deficient modified Waymouth MAB 87/3 medium for a period of 18 h. Labeled proteoglycans were isolated from the spent medium by chromatography on DEAE-Trisacryl as described (9). After addition of fetal calf serum to a final concentration of 10%, they were dialyzed against serum-free Eagle’s minimal essential medium. [35S]Sulfate-labeled glycosaminoglycan chains were liberated from the core proteins by treatment with 0.15 M NaOH for 6 h at 37 °C and used for uptake experiments after neutralization with acetic acid, addition of serum, and dialysis.

Determination of Endocytosis and Degradation of Proteoglycans—These five to seven days before endocytosis experiments the recipient cells were densely plated (60 × 15-mm culture dishes or 25-cm² plastic flasks (Falcon). Confluent cultures were preincubated for 24 h with the protease inhibitors indicated or the solvent of the drug before radioactively labeled proteoglycans were added. Preincubation and incubation media contained additionally 10 mM Hepes; the pH was adjusted to 7.5. Endocytosis and degradation were measured as described previously (6). Briefly, the radioactivity of the culture medium and of the ethanol-insoluble material in the cell pellet obtained after trypsinization was determined, as well as the ethanol-soluble radioactivity of culture medium and cell pellet. Endocytosed material represents the sum of intracellular and nonprecipitable radioactivity of the medium minus nonprecipitable radioactivity of the control medium. When expressed as clearance rate, the volume of medium cleared from labeled material/h and mg of cell protein are given. Degradation refers to the nonprecipitable part of endocytosed radioactivity.

In studies on the uptake of [3H]arginine-labeled proteoglycans recipient cells were grown in 75-cm² plastic flasks and challenged with 5 ml of medium containing [3H]arginine-labeled DS-PG. At the end of the incubation, the cell layer was washed twice with 3 ml each of 0.1% trypsin in Hanks’ salt solution and incubated with 100 μl of 0.1% trypsin for 4 min at 37 °C. 4 milliliters of Hanks’ salt solution containing 0.15 M NaCl, and 1.0 mM EDTA, disodium salt, 0.05% Nonidet P-40, 10 mM N-ethylmaleimide, and 5 mM benzamidine hydrochloride. The extract was loaded on a 1.5-ml DEAE-Trisacryl column prepared in a Pasteur pipette and equilibrated with this buffer. The column was eluted stepwise with 1.5 ml each of 0.15 M NaCl, 0.3 M NaCl, 0.4 M NaCl, and 1.0 M NaCl, all in buffer A. Appropriate fractions from the last step were dialyzed against 0.1% Triton X-100, concentrated to about 300 μl in a Speed-Vac Concentrator (Bachofen), and treated with chondroitin ABC lyase (23). The digest was dried in vacuo, washed sequentially with methanol, 20% (w/v) trichloroacetic acid, and methanol prior to SDS-polyacrylamide gel electrophoresis.

Molecular Size Analysis of Endocytosed Proteoglycans—For molecular size analysis of endocytosed proteoglycans, the cell pellet obtained after trypsinization was extracted with 400 μl of 1% SDS containing 0.1 M 6-aminohexanoic acid, 10 mM EDTA, disodium salt, 10 mM N-ethylmaleimide, and 5 mM benzamidine hydrochloride. Solubilized material prior and after the alkaline treatment described above was analyzed by high-performance liquid chromatography on a TSK G 3000 SW column (7.5 × 50 mm; Varian) in 0.1% SDS, 50 mM sodium phosphate, pH 6.0. Fractions of 0.5 ml were collected at a flow rate of 0.25 ml/min.

Subcellular Fractionation on Percoll Gradients—After endocytosis, the cell pellet postnuclear supernatant was applied on top of a Percoll gradient medium (ρ = 1.065 g/cm³) as described (24) except that the sucrose cushion was omitted. Centrifugation was for 40 min at 20,000 rpm in a VTI 50 rotor (Beckman Instruments). Fractions of 2 ml were collected and assayed for radioactivity, β-N-acetylated chondrosin, and density.

Other Methods—The dermatan sulfate-degrading enzymes β-N-acetylated chondrosin (25), α-iduronidase (26), β-glucuronidase (25), N-acetylgalactosamine-6-sulfatase (27), N-acetylgalactosamine-4-sulfatase (aryl-sulfatase E (28)), and iduramate sulfatase (29) were assayed in the absence or presence of 1 μM Cbz-Phe-Ala-CHN₂ as previously described. SDS-polyacrylamide gel electrophoresis followed by fluorography was done as quoted earlier (17). Protein was quantitated as described (29) using bovine serum albumin as standard.

RESULTS

Kinetics of Degradation of Endocytosed DS-PG—Proteoglycans secreted by human skin fibroblasts represent a mixture of various proteoglycans. 80–90% of the total amount consist of DS-PG. It had been shown previously that large chondroitin sulfate/dermatan sulfate proteoglycans are internalized by bulk phase endocytosis only (30). The clearance rate of heparan sulfate proteoglycan is about 10-fold lower than that of DS-PG (10). In consideration of the proportions of different proteoglycans about 98% of endocytosed proteoglycans reflect the uptake of DS-PG. With respect to endocytosis, the terms proteoglycan and DS-PG will, therefore, be used synonymously in the present study.

The isolation of [3H]leucine- and [35S]sulfate-labeled DS-PG made it possible to study separately the kinetics of degradation of the protein and the glycosaminoglycan moieties of endocytosed proteoglycan. Fibroblasts grown to confluency in a 75-cm² flask were challenged with DS-PG containing 2.80 × 10⁶ cpm of [3H]radioactivity and 1.55 × 10⁶ cpm of [35S]radioactivity. Aliquots of the medium were withdrawn at different times and analyzed for ethanol-soluble radioactivity while the culture was supplemented with fresh radioactive medium. Previous studies had ascertained that the ethanol-soluble radioactivity in the medium behaves like free [3H]arginine.
Degradation of Dermatan Sulfate Proteoglycan

leucine and [35S]sulfate (9). Incubation of DS-PG with conditioned medium in the absence of fibroblasts did not result in measurable degradation of the proteoglycan. Fig. 1 shows that the release of both [3H]leucine and [35S]sulfate follows parabolic curves with time. However, formation and transport to the culture medium of [3H]leucine was 10 to 15 times that of [35S]sulfate. The clearance rates (about 240 µl/h), determined at the end of the experiment, were almost identical for [3H] and [35S] radioactivity. Thus, in contrast to the protein moiety, most of the endocytosed glycosaminoglycan moiety was not degraded during the experimental period. Proteolysis of core protein, therefore, precedes the degradation of dermatan sulfate chains.

By employing longer incubation periods, it could be shown that after approximately 3 h a steady state was approached between entry of labeled DS-PG core protein into the compartment of its degradation and release of free [3H]leucine (Fig. 2C). Within 6 h no such steady state for the degradation of the dermatan sulfate chains could be observed. In the presence of a weak base, 10 mM NH4Cl, a similar time period was required to approach steady state conditions for core protein degradation (Fig. 2D), but after 3 h of endocytosis the degradation rate was only about 70% of that of the untreated control. In contrast, treatment with NH4Cl severely impaired the degradation of dermatan sulfate chains. No significant alteration of the rate of endocytosis was observed (Fig. 2A and B). Chloroquine was much more efficient than NH4Cl in inhibiting the degradation of the protein core. At a 0.1 mM concentration of the drug endocytosis was reduced to 69% in a 6-h experimental period, but liberation of free [3H]leucine was only 27% of the endocytosed amount contrasting to a value of 94% in the control.

Influence of Protease Inhibitors on the Degradation of Endocytosed DS-PG — The observation that core protein degradation precedes glycosaminoglycan degradation could point to the existence of a critical proteolytic step taking place prior to dermatan sulfate hydrolysis. We have, therefore, investigated the influence of several protease inhibitors on the degradation of endocytosed DS-PG. It is seen in Table I that two different SH-protease inhibitors (Cbz-Phe-Ala-CHN₉, E-64) affected the degradation of the glycosaminoglycan chains. The other inhibitors were ineffective, but in separate experi-

![Fig. 2. Influence of NH₄Cl on the time course of endocytosis and degradation of DS-PG. Fibroblasts in 25-cm² culture flasks were preincubated for 30 min in the presence (B, D) or absence (A, C) of 10 mM NH₄Cl and then exposed to 4.8 x 10⁴ cpm [3H]leucine- and 9.8 x 10⁴ cpm [35S]sulfate-labeled DS-PG in a total volume of 2 ml with or without NH₄Cl. ○, [3H] radioactivity; ●, [35S] radioactivity.](image-url)
TABLE I
Effect of protease inhibitors on endocytosis and degradation of [35S]sulfate-labeled DS-PG

| Addition  | Clearence  | Degradation |
|-----------|------------|-------------|
|           | μl/h/mg cell protein | % of endocytosed amount |
| None      | 106        | 82          |
| DMSO, 0.14 M | 76        | 77          |
| Cbz-Phe-Ala-CHN2, 1 μM | 90        | 22          |
| E-64, 100 μM; DMSO, 0.14 M | 87        | 34          |
| Chymostatin, 100 μM; DMSO, 0.14 M | 86        | 61          |
| Phosphoramidon, 100 μM | 89        | 77          |
| Pepstatin, 100 μM; DMSO, 0.14 M | 78        | 79          |

FIG. 3. Influence of Cbz-Phe-Ala-CHN2 on the degradation of endocytosed DS-PG. After preincubation, culture medium was supplemented with [3H]leucine and [35S]sulfate-labeled DS-PG (20,000 cpm of [3H] and 41,000 cpm of [35S] radioactivity). Endocytosis and degradation were determined after 6 h of incubation in the continuous presence of the drug. The calculated clearance rates varied between 80 and 168 μl/h and mg protein for [3H] radioactivity, and between 80 and 103 μl/h and mg protein for [35S] radioactivity. The mean protein content was 0.28 mg/dish. 84% of endocytosed [3H] radioactivity and 22% of endocytosed [35S] radioactivity had been degraded in the dimethyl sulfoxide-treated control culture. ○, [3H] radioactivity; ●, [35S] radioactivity.

FIG. 4. Time course of endocytosis and degradation of DS-PG under the influence of Cbz-Phe-Ala-CHN2. After preincubation, 33,000 cpm of [35S]sulfate-labeled DS-PG were added per plate. At the times indicated endocytosis (●, △) and degradation (○, △) were determined in control (●, ○) and Cbz-Phe-Ala-CHN2-treated (△, △) cultures.

FIG. 5. Half-life of endocytosed [35S]sulfate-labeled DS-PG. After preincubation, culture medium was supplemented with 280,000 cpm of [35S]sulfate-labeled DS-PG and 10 mM NH4Cl for 4 h. Radioactive medium was then replaced by unlabeled NH4Cl-free medium, and incubation was continued for the times indicated in the absence or presence of Cbz-Phe-Ala-CHN2. During the pulse period between 4400 and 5150 cpm were endocytosed without significant degradation, ○, intracellular radioactivity in control cultures; ●, intracellular radioactivity in Cbz-Phe-Ala-CHN2-treated cultures.

ments the cysteine- and serine-protease inhibitor leupeptin showed at a 10 μM concentration a similar inhibitory effect as 1 μM Cbz-Phe-Ala-CHN2 (result not shown).

Cbz-Phe-Ala-CHN2 was used to study the dose dependence of the drug on the degradation of endocytosed [3H]leucine- and [35S]sulfate-labeled DS-PG (Fig. 5). Unexpectedly, the degradation of the protein moiety of the proteoglycan was much less affected than the degradation of the carbohydrate portion. When fibroblasts were exposed to [3H]sulfate-labeled DS-PG and Cbz-Phe-Ala-CHN2 for up to 30 h, the proportion of degraded material on the endocytosed amount remained nearly constant (Fig. 4) indicating that the inhibitory effect did not change during this time period.

The half-life of the glycosaminoglycan moiety of endocytosed DS-PG was determined as follows. [35S]Sulfate-labeled DS-PG was endocytosed during a pulse period of 4 h in the presence of 10 mM NH4Cl. During a subsequent chase in the absence of NH4Cl the amount of intracellularly remaining radioactivity was determined (Fig. 5). A t1/2 of about 12 h was found in control cultures, whereas Cbz-Phe-Ala-CHN2 treatment resulted in a doubling of the half-life time.

Influence of Cbz-Phe-Ala-CHN2 on the Enzymatic Degradation of Free Dermatan Sulfate Chains—The inhibitory effect of SH-protease inhibitors on the degradation of endocytosed dermatan sulfate chains could theoretically be caused by a reduced activity of glycosaminoglycan-degrading hydrolases, by a delayed transport of endocytosed proteoglycan to lysosomes, or by an inaccessibility of the partially degraded substrate to the enzymes required for dermatan sulfate catab-
olism. To get an estimate of the actual activity of the enzymes required for dermatan sulfate degradation we challenged Cbz-Phe-Ala-CHN$_2$-treated fibroblasts with $[^{35}S]$sulfate-labeled dermatan sulfate which was prepared from DS-PG by $\beta$-elimination (Table II). Uptake of protein-free dermatan sulfate chains occurs most likely by bulk endocytosis only, which explains the relatively low rate of degradation within the endocytosis period of 18 h as a result of the low clearance rate. However, in several experiments Cbz-Phe-Ala-CHN$_2$ did not exert a significant influence on the catabolism of free dermatan sulfate chains.

The conclusion that dermatan sulfate-hydrolyzing enzymes were normal after Cbz-Phe-Ala-CHN$_2$ treatment was supported by in vitro assays of $\beta$-glucuronidase, $\alpha$-L-iduronidase, $\beta$-N-acetylhexosaminidase, iduronide 2-sulfatase, and N-acetylgalactosaminidase. Maximal activity of the enzyme was found in fractions 17 and 18.

**Transport of DS-PG to Lysosomes**—The second possibility to explain the inhibitory effect of SH-protease inhibitors, delayed transport of endocytosed DS-PG to lysosomes, has been investigated by subcellular fractionation of fibroblasts after various time periods of endocytosis (Fig. 6). At least within 3 h similar amounts of radioactive material could be detected in the region of dense lysosomes (density approximately 1.08 g/cm$^3$) in control and Cbz-Phe-Ala-CHN$_2$-treated fibroblasts. As expected, there was accumulation of radioactivity in dense lysosomes during the subsequent endocytosis period in treated cultures. Only minor amounts of radioactivity were detected in the region of light lysosomes (density approximately 1.05 g/cm$^3$). This fraction represents a heterogeneous mixture of microsomal vesicles including endosomes (31). Its content of $^{35}S$SO$_4$ radioactivity was similar in preparations from treated and control cultures. It should also be noted that only minor amounts of radioactivity were found in the nuclear pellets.

**Molecular Size of Endocytosed DS-PG**—To test for the accessibility of endocytosed DS-PG for DS-PG-degrading enzymes fibroblasts were allowed to take up labeled proteoglycan in the presence of 10 mM NH$_4$Cl during a 4-h pulse, and the molecular size of endocytosed material was followed during a subsequent chase in the absence of NH$_4$Cl. Cell extracts were chromatographed on a TSK 3000 SW column prior and after a $\beta$-elimination reaction (Fig. 6). Within the first 2 h of chase, there was a shift of the peak of the radioactivity in the control culture; thereafter only peak broadening could be observed. Peaks of progressively lower molecular size have not been

### Table II

**Effect of Cbz-Phe-Ala-CHN$_2$ on endocytosis and degradation of dermatan sulfate chains**

| Endocytosis substrate | Cbz-Phe-Ala-CHN$_2$ | Clearance | Degradation
|-----------------------|--------------------|-----------|-----------
| Dermatan sulfate      | 36                 | 41        |           |
| Dermatan sulfate      | 31                 | 36        |           |
| DS-PG                 | 156                | 64        |           |
| DS-PG                 | 167                | 32        |           |

### Table III

**Effect of cycloheximide on endocytosis and degradation of $[^{35}S]$sulfate-labeled DS-PG**

Fibroblasts were pretreated with 20 $\mu$M cycloheximide or 1 $\mu$M Cbz-Phe-Ala-CHN$_2$ for the times indicated and then incubated with DS-PG (68,000 cpm) for 12 h in the continuous presence of the drugs.

| Pretreatment   | Clearence | Degradation |
|----------------|-----------|-------------|
| None           | 54        | 54          |
| Cycloheximide  | 66        | 66          |
| 24 h           | 73        | 63          |
| Cbz-Phe-Ala-CHN$_2$ | 50 | 32          |
| 24 h           | 50        | 12          |

Fig. 6. Subcellular fractionation of Cbz-Phe-Ala-CHN$_2$-treated and control fibroblasts. After preincubation, the cultures were challenged with 60,000 cpm of $[^{35}S]$sulfate-labeled DS-PG. At the times indicated the cells were homogenized, and the postnuclear supernatant was subjected to fractionation on a Percoll gradient. The fractions were analyzed for radioactivity (O, Cbz-Phe-Ala-CHN$_2$-treated cells; O, control cells), density (broken line), and $\beta$-N-acetylhexosaminidase. Maximal activity of the enzyme was found in fractions 17 and 18.
found. In Cbz-Phe-Ala-CHN$_2$-treated cultures, the shift of the radioactivity peak was delayed. The size of the glycosaminoglycan chains was almost identical in extracts from untreated and treated cells after chase periods of 2 and 4 h; a maximum difference of 3% was found when the radioactivity of a given fraction was expressed as percent of total radioactivity. It is, therefore, likely that the size differences shown in Fig. 7 result from differences in the proteolytic degradation of the core protein.

**Intracellular Accumulation of DS-PG Peptides**—The results described in the preceding sections suggest that DS-PG peptides are accumulating in Cbz-Phe-Ala-CHN$_2$-treated cultures. Since the sequence around serine 4, the attachment site of the dermatan sulfate chain, is rich in arginine residues (20) and since the most prominent lysosomal SH-protease, cathepsin B, is very active toward arginine carboxyl bonds (32), [H]$^3$H arginine-labeled DS-PG was used to search for such peptides. Fig. 8 shows that after endocytosis of this proteoglycan only trace amounts of dermatan sulfate peptides could be isolated from the control culture. In contrast, an only partially degraded DS-PG species was found in the presence of the thiol protease inhibitor.

**DISCUSSION**

The results described in this paper provide evidence that during the course of intracellular degradation of endocytosed DS-PG proteolysis of the core protein precedes the breakdown of the dermatan sulfate chains. Release of inorganic sulfate from DS-PG represents a measure of polysaccharide degradation since in fibroblasts hyaluronoglucosaminidase is absent (33, 34) and only exoglycosidases and exosulfatases are involved in dermatan sulfate catabolism (3). The time course of the degradation of endocytosed DS-PG suggests that high molecular peptidoglycans are accumulating as intermediary products. Oligosaccharides smaller than decasaccharides and inorganic sulfate are not accumulating intracellularly. The half-life (about 12 h) of the glycosaminoglycan moiety of endocytosed DS-PG is similar to that of total [H]$^{35}$S sulfate-labeled intracellular glycosaminoglycans in fibroblasts (1). Evidence for the existence of two kinetically distinct pathways as shown for DS-PG degradation in rat ovarian granulosa cells (14) has not been obtained.

An unexpected finding of this study was the observation that SH-protease inhibitors have a much more dramatic influence on the dermatan sulfate than on the core protein degradation of endocytosed DS-PG. Leupeptin has been shown by Yanagishita (35) to slow down the degradation of cell-associated heparan sulfate proteoglycans in one of the two kinetically distinct degradation pathways in rat ovarian granulosa cells. The main inhibitory effect of leupeptin, however, was considered to result from the interference with the translocation of glycosaminoglycans to the final degradation site. In Cbz-Phe-Ala-CHN$_2$-treated fibroblasts transport of endocytosed DS-PG to dense lysosomes was apparently not delayed, and there was no indication of DS-PG accumulation in a nonlysosomal compartment which could have been caused by the drug. Several lines of evidence indicated that Cbz-Phe-Ala-CHN$_2$ did not interfere with the activity of dermatan sulfate-degrading enzymes. All enzymes known to be involved in dermatan sulfate catabolism exhibited normal activities in vitro. Furthermore, the degradation of endocytosed free dermatan sulfate chains was unaffected by the protease inhibitor.

The latter observation also supports the conclusion of an unaltered endocytotic transport, albeit different routes of transport of endocytosed DS-PG and of free dermatan sulfate cannot be excluded (36).
The most plausible interpretation of our data is, therefore, the assumption that the inhibition of a critical proteolytic step leads to an inaccessibility of partially degraded DS-PG for dermatan sulfate-degrading enzymes. It would be conceivable that the dissociation of the complex between DS-PG and its putative receptor involves the action of an SH-protease. Upon inhibition of this step receptor-bound DS-PG would arrive in lysosomes, and degradation would be impaired as the proteoglycan is still associated with the lysosomal membrane. However, the kinetics of DS-PG endocytosis suggest a reutilization of DS-PG receptors (8). The lack of an inhibition of DS-PG uptake by cycloheximide treatment for 24 h is also inconsistent with this hypothesis. It seems, therefore, more likely that the very basic DS-PG core protein (pI 9.8, Ref. 20) interacts with the single polyanionic dermatan sulfate chain attached to serine 4 at the N terminus of the core protein (19) in a fashion that renders the glycosaminoglycan chains largely inaccessible for the degrading exoenzymes. This hypothesis implies that extensive proteolysis has to precede dermatan sulfate degradation and, furthermore, that the inhibition of some critical proteolytic steps may lead to a reduced glycosaminoglycan breakdown as a secondary effect.

The inhibition of dermatan sulfate degradation under the influence of Cbz-Phe-Ala-CHN, does not concern endocytosed DS-PG only. It had been shown previously that fibroblasts divert a portion of proteoglycans to the lysosomes immediately after biosynthesis (1). Upon treatment with the protease inhibitor there is a 2-fold intracellular accumulation of [35S]sulfate-labeled proteoglycans during a 24-h pulse experiment resembling the behavior of mucopolysaccharidosis fibroblasts. Whether treatment with the drug could serve as a mucopolysaccharidosis model, remains to be investigated.

Acknowledgment — The excellent technical assistance of P. Blumberg is gratefully acknowledged.

REFERENCES
1. Fratantoni, J. C., Hall, C. W., and Neufeld, E. F. (1968) Proc. Natl. Acad. Sci. U. S. A. 60, 599–706
2. McKusick, V., and Neufeld, E. F. (1983) in The Metabolic Basis of Inherited Disease (Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L., and Brown, M. S., eds) 5th Ed., pp. 751–771, McGraw-Hill Publications, Minneapolis, MN
3. Kresse, H., and Glossl, J. (1987) in Advances in Enzymology (Meister, A., ed.) Vol. 80, pp. 217–311, John Wiley & Sons, New York
4. Diment, S., and Stahl, P. (1986) J. Biol. Chem. 260, 15311–15317
5. Schaudies, R. P., Gorman, R. M., Savage, C. R., Jr., and Poretz, R. D. (1987) Biochem. Biophys. Res. Commun. 143, 710–715
6. Kresse, H., Tekof, W., von Figura, K., and Buddecke, E. (1975)

Hoppel Seyler’s Z. Physiol. Chem. 356, 943–952
7. Trupke, W., and Kresse, H. (1978) Eur. J. Biochem. 85, 351–356
8. Prinz, R., Schwermann, J., Buddecke, E., and von Figura, K. (1978) Biochem. J. 176, 671–676
9. Glossl, J., Schwabert-Prinz, R., Gregory, J. D., Darke, S. P., von Figura, K., and Kresse, H. (1986) Biochem. J. 231, 295–301
10. Kruger, U., and Kresse, H. (1986) Biochim. Biophys. Acta 867, 465–471
11. Smersdor, B., Kjellen, L., and Pertot, H. (1985) Biochim. J. 229, 63–71
12. Laurent, T. C., Fraser, R. E., Pertot, H., and Smersdor, B. (1986) Biochem. J. 234, 653–658
13. Volk, W., Schmidt, A., Robenek, H., and Buddecke, E. (1984) Eur. J. Cell Biol. 34, 110–117
14. Yanagishita, M., and Hascall, V. C. (1984) J. Biol. Chem. 259, 10270–10280
15. Ishihara, M., Fedarko, N. S., and Balschak, E. (1983) J. Biol. Chem. 261, 13575–13580
16. Höök, M., Kjellen, L., and Johannsen, S. (1984) Annu. Rev. Biochem. 53, 847–869
17. Glossl, J., Beck, M., and Kresse, H. (1985) J. Biol. Chem. 259, 14144–14150
18. Rosenberg, L. C., Choi, H. U., Tang, L.-H., Johnson, T. L., Pal, S., Webber, C., Reiner, A., and Poole, A. R. (1985) J. Biol. Chem. 260, 6304–6313
19. Chopra, R. K., Pearson, C. H., Pringle, G. A., Fackre, D. S., and Scott, P. G. (1986) Biochem. J. 232, 277–279
20. Krussius, T., and Roushakhi, E. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7683–7687
21. Shaw, E., and Green, G. D. J. (1981) Methods Enzymol. 80, 820–826
22. Cantz, M., Kresse, H., Barton, R. W., and Neufeld, E. F. (1972) Methods Enzymol. 28, 884–897
23. Saito, H., Yanagata, T., and Suzuki, S. (1968) J. Biol. Chem. 243, 1536–1542
24. Giesemann, V., Pohlmann, R., Hasilik, A., and von Figura, K. (1983) J. Cell Biol. 97, 1–5
25. von Figura, K. (1978) Eur. J. Biochem. 80, 525–533
26. Liebaers, I., and Neufeld, E. F. (1973) Pediat. Res. 10, 733–736
27. Kresse, H., von Figura, K., Klein, U., Glossl, J., Paschke, E., and Pohlmann, R. (1985) Methods Enzymol. 83, 559–572
28. Steckel, F., Hasilik, A., and von Figura, K. (1985) Eur. J. Biochem. 151, 141–146
29. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
30. von Figura, K., Mittelviehhaus, H., Prinz, R., Duchene, M., and Krieg, T. (1981) in Biology of the Articular Cartilage in Health and Disease (Gastpar, H., ed.) pp. 189–195, F. K. Schattauer Verlag, Stuttgart
31. Berg, T., Kindberg, G. M., Ford, T., and Blomhoff, R. (1985) Exp. Cell Res. 161, 286–296
32. Katunuma, N., Towatari, T., Tamai, M., and Hanada, K. (1983) J. Biochem. (Tokyo) 93, 1129–1135
33. Arbogast, B., Hopwood, J. J., and Dorfman, A. (1975) Biochem. Biophys. Res. Commun. 67, 376–382
34. Klein, U., and von Figura, K. (1980) Biochim. Biophys. Acta 630, 10–14
35. Yanagishita, M. (1985) J. Biol. Chem. 260, 11075–11082
36. Wileman, T., Harding, C., and Stahl, P. (1985) Biochem. J. 232, 1–14

2 H. Kresse, unpublished observation.