Receptor-mediated endocytosis of proteoglycans by human fibroblasts involves recognition of the protein core

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Endocytosis by cultured human skin fibroblasts of $^{35}\text{SO}_4^{2-}$ -labelled or [^3H]leucine-labelled proteoglycans from fibroblast secretions and of ^{125}I -proteodermatan sulphate from pig skin was quantitatively investigated. The following results were obtained. (1) Core proteins prepared by digestion with chondroitin ABC lyase were at least as efficiently endocytosed as native proteoglycans. Pig skin proteodermatan sulphate was a competitive inhibitor of endocytosis of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans. (2) Proteoglycans produced in the presence of tunicamycin and native proteoglycans degraded with endoglycosaminidase H were internalized at a normal rate. Several monosaccharides that can be bound by mammalian lectins were unable to influence the internalization of proteoglycans. Treatment of proteoglycans with neuraminidase, however, resulted in an increased clearance rate. (3) Reductive methylation or acetoacetylation of lysine residues was accompanied by a parallel decrease in the rate of proteoglycan endocytosis. Reversal of acetoacetylation normalized the uptake properties. Endocytosis of native proteoglycans was also reduced in the presence of poly-L-lysine, and this reduction in endocytosis was observed as well with proteoglycans synthesized in the presence of the lysine analogue *S*-2-aminoethylcysteine. These results suggest that the recognition marker required for receptor-mediated endocytosis of proteodermatan sulphate resides in its protein moiety and involves lysine residues.

Cultured human skin fibroblasts synthesize several types of proteoglycans, proteodermatan sulphate being quantitatively the most important one (Malmström *et al.*, 1975; Sjöberg *et al.*, 1979; Cöster *et al.*, 1979; Carlstedt *et al.*, 1981). The bulk of it is secreted into the culture medium. Proteoglycans present in the extracellular compartment may reversibly associate with the cell surface and may become internalized by endocytosis. Uptake and subsequent transfer to lysosomes is required before the glycosaminoglycan chains can be degraded enzymically.

It has been shown previously that endocytosis of proteodermatan sulphate by human skin fibroblasts is a highly efficient, specific and saturable process as has been proposed for receptor-mediated pinocytosis (Neufeld & Cantz, 1973; Truppe & Kresse, 1978; Prinz *et al.*, 1978). For receptor-mediated endocytosis macromolecules must be provided with

a recognition marker that interacts with cell-surface receptors. Since free glycosaminoglycan chains did not inhibit (Truppe & Kresse, 1978), or inhibited only non-competitively (Prinz *et al.*, 1978), the uptake of proteoglycans, it was concluded that the protein core of proteoglycans is essential for binding to the receptor. However, the potential role of *N*- and *O*-glycosidically linked oligosaccharides, which are components of proteodermatan sulphate from skin (Damle *et al.*, 1982), in the interaction with the receptor has not yet been studied. The situation is further complicated by the observation that Chinese hamster cells are able to internalize protein-free chondroitin sulphate in a saturable manner (Saito & Uzman, 1971). Glycosaminoglycans synthesized on xylose or *p*-nitrophenyl β -D-xyloside as initiators of chain formation in place of protein core were also taken up by human fibroblasts, albeit less efficiently than proteoglycans (Truppe & Kresse, 1978).

The present paper reports evidence that the recognition marker for uptake of proteodermatan sulphate is localized in the protein moiety.

Experimental

Materials

The following substances were purchased from the suppliers indicated: $\text{Na}_2^{35}\text{SO}_4$ (carrier-free), L-[4,5(n)- ^3H]leucine (sp. radioactivity, 59.8 Ci/mmol) and Na^{125}I (carrier-free) from Amersham-Buchler, Braunschweig, Germany; DEAE-Trisacryl from LKB Instrument, Gräfelfing, Germany; DE-52 DEAE-cellulose from Whatman, Maidstone, Kent, U.K.; chondroitin ABC lyase (EC 4.2.2.4), tunicamycin, mannose 6-phosphate, S-2-aminoethyl-cysteine and poly-L-lysine (mol.wt. 17000) from Sigma, Munich, Germany; endo- β -N-acetylglucosaminidase H (EC 3.2.1.96) from Miles, Frankfurt/Main, Germany; neuraminidase from *Vibrio cholerae* (EC 3.2.1.18) from Behringwerke, Marburg, Germany. Lactoperoxidase (80 units/mg) from Sigma was coupled with CNBr-Sepharose (Pharmacia, Freiburg, Germany) according to the instructions of the manufacturer. The preparation and characterization of proteodermatan sulphate from pig skin was as described previously (Damle *et al.*, 1982).

Cell culture

Human skin fibroblasts were grown in 75 cm² Falcon plastic flasks in modified Eagle's minimum essential medium (Eagle, 1959), supplemented with 10% (v/v) foetal-calf serum (LS-Labor Service, Munich, Germany), non-essential amino acids and antibiotics, as described by Cantz *et al.* (1972). Two days before uptake experiments, cells were densely plated in 35 mm $\times$ 14 mm multiwell plates (Becton & Dickinson, Basel, Switzerland).

Preparation of proteoglycans and protein cores

At 1 h before labelling, Eagle's minimum essential medium was replaced by 5 ml of Waymouth MAB 87/3 medium (Gorham & Waymouth, 1965)/75 cm² Falcon plastic flask, as formulated in the catalogue of GIBCO (Grand Island, NY, U.S.A.), in which MgSO_4 had been replaced by MgCl_2 . The labelling medium, 4.4 ml, was the same deficient medium, supplemented with 0.2 ml of dialysed foetal-calf serum (Flow Laboratories, Munich, Germany) and 0.1 mCi of $\text{Na}_2^{35}\text{SO}_4$. In some experiments, leucine-free Waymouth medium was used, and 0.2 mCi of [^3H]leucine was added.

At the end of the incubation period, usually after 24 h, medium was made 70% saturated with $(\text{NH}_4)_2\text{SO}_4$. After centrifugation, the precipitate was dissolved in 5 ml of 20 mM-Tris/HCl buffer, pH 7.5,

containing 0.1 M-6-aminohexanoic acid, 10 mM-EDTA (disodium salt), 10 mM-N-ethylmaleimide, 5 mM-benzamidine hydrochloride (buffer A) and 0.15 M-NaCl and dialysed against the same buffer. The dialysis residue was then loaded on a DEAE-Trisacryl column (0.7 cm $\times$ 13 cm) equilibrated with this buffer. The column was eluted stepwise with 15 ml each of 0.15 M-NaCl, 0.3 M-NaCl and 1.0 M-NaCl, all in buffer A. Appropriate fractions from the last step were pooled. Foetal-calf serum was added to a final concentration of 10% before dialysis against serum-free Eagle's minimum essential medium.

For preparation of [^3H]leucine-labelled protein cores proteinase inhibitors were omitted in the last elution step of the DEAE-Trisacryl column. Fractions were dialysed against 10 mM-Tris/HCl buffer, pH 7.4, containing 0.15 M-NaCl and digested with 20 m-units of chondroitin ABC lyase for 4 h at 37°C in a final volume of 200 μl (Saito *et al.*, 1968). Controls were incubated with proteinase inhibitors (Oike *et al.*, 1980) or without enzyme. After addition of complete tissue culture medium, the digest was directly used for uptake experiments.

Proteoglycan modifications

For amino acid modifications $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans in 0.15 M-NaCl were used. Lysine residues were methylated and acetoacetylated by the method of Weisgraber *et al.* (1978). For reductive methylation 800 μl of proteoglycan solution (120 000 c.p.m.) were mixed at 0°C with 8 mg of bovine serum albumin and 400 μl of 0.3 M-sodium borate buffer, pH 9.0. NaBH_4 (2 mg) and 2 μl of 35% (w/v) formaldehyde were added at zero time. Addition of formaldehyde was repeated after 6, 12, 18, 24 and 30 min. After a further 6 min, 6 mg of NaBH_4 was added, followed by 24 μl of 2 M-acetic acid 4 min later. In controls, formaldehyde and NaBH_4 were omitted.

For acetoacetylation 400 μl of proteoglycan solution (425 000 c.p.m.), 1 mg of bovine serum albumin, 200 μl of 0.3 M-sodium borate buffer, pH 8.5, and 15 μl of 60 mM-diketene prepared in 0.1 M-sodium borate, pH 8.5, were allowed to react for 5 min at 22°C before addition of 40 μl of ethanol. After 2 h, the mixture was dialysed for 6 h against 10 mM-Tris/HCl buffer, pH 7.0, containing 0.15 M-NaCl. For reversal of lysine diketene modification 250 μl of the dialysis residue was treated with 100 μl of 1.5 M-hydroxylamine/HCl, pH 7.0, for 14 h at 37°C. Controls were incubated without diketene.

For selective modification of arginine residues (Mahley *et al.*, 1980), 200 μl of proteoglycan solution (85 000 c.p.m.) was incubated with 1 mg of bovine serum albumin and 400 μl of 0.15 M-cyclohexane-1,2-dione in 0.2 M-sodium borate buffer, pH 8.1, for

2 h at 37°C. Cyclohexanedione was omitted in controls.

For digestion of proteoglycans with endoglucosaminidase H 0.25 ml of proteoglycan solution in 0.15 M-NaCl was mixed with 50 µl of 0.3 M-sodium phosphate buffer, pH 5.5, and 0.1 unit of enzyme and incubated for 24 h at 37°C.

At the end of each modification reaction portions of the samples were made 10% with respect to foetal-calf serum and dialysed against serum-free Eagle's minimum essential medium. A second portion of the samples was dialysed against 0.15 M-NaCl and then used for amino acid analysis.

Iodination of proteodermatan sulphate from pig skin and of its protein core was carried out by a modification of the method of Marchalonis (1969). Protein (1 mg) in 110 µl of 0.01 M-sodium phosphate buffer, pH 7.0, containing 0.1 M-NaCl was mixed with 11 µl of Sepharose-linked lactoperoxidase in the same buffer, 1 µl of 1 mM-H₂O₂ and 1 mCi of Na¹²⁵I, final volume 133 µl. After 10 min at 22°C the reaction was terminated by adding 5 µl of 1 M-Na₂S₂O₃ and 10 mg of KI. Iodinated product was purified from the supernatant by chromatography on a Sephadex G-25 column (1.5 cm × 25 cm) equilibrated with 10 mM-sodium phosphate buffer, pH 7.0, containing 0.1 M-NaCl, 3 mM-Na₂S₂O₃ and 1 g of bovine serum albumin/litre. The material eluting in the void volume passed unadsorbed through a 1.5 ml DEAE-cellulose column equilibrated with a 50 mM-sodium phosphate buffer, pH 7.0. The specific radioactivity was 3×10^7 c.p.m./mg of protein.

Determination of endocytosis, degradation and adsorption

Endocytosis, degradation of endocytosed material and adsorption of exogenously added compounds to the cell surface were determined in duplicate assays as described previously (Kresse *et al.*, 1975; Prinz *et al.*, 1978). Briefly, the radioactivity of the culture medium, of the material removable by trypsin from the cell membrane and of the ethanol-insoluble material in the cell pellet was measured, as well as the ethanol-soluble radioactivity of the culture medium and cell pellet. In the case of labelled core proteins ethanol precipitation was replaced by precipitation with 0.3 M-trichloroacetic acid for 1 h at 0°C. Uptake was defined as the sum of intracellular and non-precipitable radioactivity of the medium minus non-precipitable radioactivity of the control medium. It is expressed as clearance rate, giving the volume of medium (in µl) cleared from labelled material/h per mg of cell protein. The non-precipitable part of the radioactivity taken up represents the amount of degraded material. Adsorption is referred to as the amount of radioactivity found in the trypsin solution.

The previously published protocol was modified

as follows. Confluent monolayers in 35 mm × 14 mm multiwell plates were used; the total volume of medium/dish was 1.5 ml; the concentration of labelled material varied between 30 000 and 90 000 c.p.m./ml; uptake was routinely measured after 6 h of incubation, but occasionally after 22 h. Variation of the incubation period had no influence on the clearance rate and the amount of adsorbed material. Within an experimental series the results of duplicate incubations differed by approx. 10%. The phenomena to be described were reproducibly observed in separate experiments though considerable variation was noted when the clearance rates of different series were compared. These differences could not be explained by variation of the cell density and of the pH of the culture medium (Prinz *et al.*, 1978).

Other methods

The purity of proteoglycans and their core proteins was judged by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate [Laemmli (1970), as modified by Hasilik & Neufeld (1980)] followed by fluorography (Bonner & Laskey, 1974). For direct radioactivity measurements gel slices were solubilized in 30% H₂O₂/1 M-NH₃ (100:1, v/v) (Goodman & Matzura, 1970). Glucuronic acid (Bitter & Muir, 1962), lysine (Habeeb, 1966), arginine (Davies *et al.*, 1972), cell protein (Kaltwasser *et al.*, 1965) and β-N-acetylhexosaminidase (von Figura, 1977) were determined as described.

Results and discussion

Recognition of proteodermatan sulphate core proteins

³⁵SO₄²⁻-labelled proteoglycans secreted by human skin fibroblasts represent a mixture of a glucuronic acid-rich proteodermatan sulphate (approx. 5% of total radioactivity), which elutes with the void volume of a Sepharose CL-4B column, an iduronic acid-rich proteodermatan sulphate (approx. 85% of total radioactivity) of smaller molecular size and proteoheparan sulphate (approx. 10% of total radioactivity; M. Beck, J. Glössl, R. Rüter & H. Kresse, unpublished work). Previous studies have shown that dermatan sulphate-rich proteoglycans are at least three times more efficiently internalized than proteoheparan sulphate (Kresse *et al.*, 1975). Since proteoheparan sulphate represents only a small proportion of the proteoglycan preparation and does not contribute significantly to the amount of internalized proteoglycans the terms proteoglycan and proteodermatan sulphate will be used synonymously in the present study.

Initial attempts to characterize the part of the proteoglycan molecules mediating recognition and

Fig. 1. Double-reciprocal plot of endocytosis of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans against proteoglycan concentration in the absence (O) and presence (●) of pig skin proteodermatan sulphate (80 μmol of disaccharide unit/litre)

Uptake was measured after 6 h of incubation; the mean protein content was 0.2 mg/dish. Each symbol represents the mean of duplicate determinations; bars indicate the range of data when the latter exceeded the size of symbols.

subsequent endocytosis by fibroblasts were hampered by the fact that proteoglycans from fibroblast secretions lost their uptake properties upon purification. We searched therefore for competitive inhibitors of endocytosis of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans. Proteodermatan sulphate from pig skin (Damle *et al.*, 1982) inhibited receptor-mediated endocytosis of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans in a competitive manner with a K_i of 12.9 μmol of disaccharide unit/litre (Fig. 1). In the absence of the inhibitor half-maximal uptake was achieved at a concentration of 11.8 μmol of disaccharide unit/litre. This suggests that both types of proteoglycan competed for the same cell surface binding sites. Iodinated pig skin proteodermatan sulphate was internalized with a similar efficiency to that of proteoglycans from fibroblast secretions (Table 1). ^{125}I -Labelled core proteins prepared from unlabelled or iodinated pig skin proteodermatan sulphate by exhaustive digestion with chondroitin ABC lyase were internalized with an efficiency comparable with that of the parent molecules. In the presence of a 60-fold excess of unlabelled protein core or parent pig skin proteoglycan uptake of ^{125}I -labelled protein core was reduced to 57% and 67% of the control value respectively (results not shown). These results led to the conclusion that the recognition marker for uptake of pig skin proteodermatan sulphate (and by analogy for proteoglycans secreted by human

Table 1. Clearance of proteoglycans and of proteodermatan sulphate core proteins

In Expt. 1 approx. 50000 c.p.m. was added per plate; the incubation period was 22 h; the mean protein content was 0.27 mg/dish. In Expt. 2 30000 c.p.m. was added to plates of the same cell density; the incubation period was 6 h.

Expt. no.	Preparation	Clearance ($\mu\text{l/h}$ per mg of cell protein)	Degradation (% of endocytosed amount)
1	Pig skin ^{125}I -proteodermatan sulphate	49.0	99
	^{125}I -labelled core protein from pig skin proteodermatan sulphate	44.0	98
	^{125}I -labelled core protein iodinated after preparation of core protein	49.5	98
	$^{35}\text{SO}_4^{2-}$ -labelled proteoglycans	50.1	80
	[^3H]Leucine-labelled proteoglycans	30.3	96
2	[^3H]Leucine-labelled core proteins	37.2	96

fibroblasts) is part of the protein core and does not reside in the glycosaminoglycan moiety of the molecule. Furthermore, the efficiency of uptake of proteoglycans from different mammalian species appears to be similar, as has been suggested previously (Bleckmann & Kresse, 1980).

Recent progress in purification and preparation of protein cores from secreted proteoglycans under conditions preserving the uptake properties allowed us to identify the recognition marker of secreted proteoglycans as part of the protein core. The gel-electrophoretic characterization of proteoglycans and protein cores prepared from the secretions of fibroblasts is shown in Fig. 2. [^3H]Leucine-labelled proteoglycans migrated as a diffuse band with a slower mobility than phosphorylase *b* upon gel electrophoresis in the presence of sodium dodecyl sulphate. Upon degradation with chondroitin ABC lyase 90% of the total radioactivity appeared as protein bands with M_r 48000 and 45000 respectively. The M_r -48000 band contained 1.9 (1.6–2.1; $n = 5$) times the radioactivity of the smaller one. These bands are considered to represent proteodermatan sulphate core proteins. It is not yet clear whether they are genetically distinct or whether they arise as a result of different post-translational processing. In contrast with the findings of Carlstedt *et al.* (1981) we were unable to detect a proteodermatan sulphate core protein of $M_r > 400000$.

Uptake of [^3H]leucine-labelled proteodermatan sulphate and of its core proteins was compared

Fig. 2. Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate of proteodermatan sulphate and its protein cores

A linear gradient from 5% to 15% (w/v) of total monomer concentration was used in the separation gel. Track (a), purified [^3H]leucine-labelled proteoglycans; track (b), the same material after digestion with chondroitin ABC lyase; track (c), chondroitin ABC lyase digest of the material remaining in the culture medium after endocytosis of 19.6% of added ^3H -labelled proteoglycan.

Table 2. Effect of oligosaccharide modification and influence of saccharides on endocytosis and degradation of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans

In Expts. 1 and 2 approx. 30000 c.p.m. was added per plate; the mean protein content was 0.21 mg/dish. In Expt. 3 120000 c.p.m. was added per plate; the mean protein content was 0.26 mg/dish; with the exception of mannose 6-phosphate saccharides were added as 300 mM solutions in a volume one-tenth the final volume; the final concentration of mannose 6-phosphate was 2 mM. In Expt. 4 42000 c.p.m. was added per plate; the protein content was 0.15 mg/dish; galactose was added to give a concentration of 30 mM. The incubation period was 6 h in all experiments.

Expt. no.	Treatment or addition	Clearance ($\mu\text{l/h}$ per mg of protein)	Degradation (% of endocytosed amount)
1	Tunicamycin	34.7	82
	Control	35.4	76
2	Endoglucosaminidase H	27.8	84
	Control	28.2	82
3	Saccharose	15.2	97
	Mannose	15.7	98
	Galactose	18.7	98
	Fucose	17.4	98
	<i>N</i> -Acetylglucosamine	18.0	98
	<i>N</i> -Acetylgalactosamine	16.2	96
	Mannose 6-phosphate	15.4	97
4	Control	18.6	97
	Neuraminidase	26.0	82
	Neuraminidase + galactose	26.0	86
	Control	21.2	86

Effect of saccharides on endocytosis

Proteodermatan sulphate core proteins bear *N*- and *O*-glycosidically linked sugar residues, which might mediate the binding to cell-surface lectins (for review, see Neufeld & Ashwell, 1980).

The presence of various monosaccharides, saccharose and mannose 6-phosphate was without effect on uptake of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans (Table 2). Treatment of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans with neuraminidase, however, facilitated their subsequent endocytosis. This effect seemed not to be due to the exposure of terminal galactose residues, since simultaneous addition of 30 mM-galactose did not reduce their uptake (Table 2).

Removal of high-mannose-type oligosaccharides by treatment with endo- β -*N*-acetylglucosaminidase H did not alter the uptake properties of proteoglycans. In a control experiment it has been proven that treatment of [^3H]leucine-labelled proteodermatan sulphate core proteins with this enzyme results in the conversion of about half the amount of the M_r 48000 core protein to a protein of lower molecular size. In a second set of experiments

(Table 1). The core proteins became even more efficiently internalized than the native proteoglycan. No preference for endocytosis of one of the core proteins could be detected since the same electrophoretic pattern of the proteins remaining in the culture medium at the end of the endocytosis experiment was found (Fig. 1). Chondroitin ABC lyase added together with the core proteins did not influence the rate of internalization. When monolayers were pretreated with a corresponding amount of enzyme for up to 6 h before addition of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans (100000 c.p.m./dish), no significant difference in the clearance rate of treated and untreated cultures was found after 1 h of incubation.

$^{35}\text{SO}_4^{2-}$ -labelled proteoglycans were prepared from secretions of fibroblasts that had been pre-incubated for 16 h in the presence of $1.2\ \mu\text{M}$ -tunicamycin, an inhibitor of *N*-glycosylation, and then labelled with $\text{Na}_2^{35}\text{SO}_4$ for 3 days in the presence of the drug. Incorporation of $^{35}\text{SO}_4^{2-}$ into secreted proteoglycans was reduced to 7% of the control, which had been treated with the solvent dimethyl sulphoxide (3.5 mM) alone. Proteoglycans from tunicamycin-treated cultures were as efficiently endocytosed as those from control cultures (Table 2). These results indicate that the protein rather than the oligosaccharide moiety of the proteodermatan sulphate core proteins bears the recognition marker for the membrane receptor.

The mechanism of the tunicamycin-mediated reduction of the secretion of proteoglycans has not been evaluated in the present study. Pratt *et al.* (1979) found tunicamycin to inhibit the synthesis of proteoglycans in cultured chick embryo fibroblasts. The effect could be overcome by the addition of β -xylosides, suggesting that the synthesis or processing of core proteins may be altered, or the activity of the xylosyltransferase may be decreased.

Effect of amino acid modifications on endocytosis

Experiments to modify lysine and arginine residues of $^{35}\text{SO}_4^{2-}$ -labelled proteodermatan sulphate were carried out as described in the Experimental section. The limited amount of core protein in the sample did not allow quantification of unmodified amino acids; instead, albumin was added as carrier, and amino acids were determined in the incubation mixture. In spite of this limitation, there was good correlation between the extent of modification of lysine residues and the reduction of the clearance rate (Table 3). Cyclohexanedione modification of arginine residues produced a less pronounced effect.

Though the modification procedures were shown to be selective and mild (Weisgraber *et al.*, 1978), the results cannot be interpreted unequivocally, since the consequences of the modification for the conformation of the core proteins are unknown. Reductive methylation, however, does not alter the charge of the protein. Nevertheless, it leads to a decreased clearance rate. Reversal of diketene modification also reverses the effect on uptake though the treatment with hydroxylamine at 37°C itself decreases the clearance rate to a certain extent.

The finding that lysine residues of core proteins are of importance for adsorptive endocytosis of proteodermatan sulphate is supported by the observation that poly-L-lysine acts as an inhibitor of uptake in a dose-dependent manner (Table 4). Association of proteoglycans with the cell membrane was greatly facilitated even when small doses of poly-L-lysine were added. It seems likely that this

Table 3. *Effect of modification of lysine and arginine residues on endocytosis of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans* 46 000–58 000 c.p.m. was added per plate. The mean cellular protein content was 0.17 mg/dish. The incubation period was 6 h. Values in parentheses represent percentages of the respective controls.

Modification	Clearance ($\mu\text{l/h}$ per mg of cell protein)	Degradation (% of endo- cytosed amount)	Unmodified amino acid (%)
Reductive methylation	3.8 (17)	84	14
Control	22.7	92	100
Acetoacetylation	10.7 (51)	81	51
Control	21.1	91	100
Acetoacetylation, reversed	16.3 (92)	91	91
Control	17.7	90	100
Cyclohexanedione modification	16.2 (71)	88	45
Control	22.7	90	100

Table 4. *Influence of poly-L-lysine on endocytosis, degradation and adsorption of $^{35}\text{SO}_4^{2-}$ -labelled proteoglycans* Approx. 35 000 c.p.m. (4 nmol of glucuronic acid) was added per plate; the mean protein content was 0.12 mg/dish. Uptake was measured after 6 h of incubation.

Concentration of poly- L-lysine ($\mu\text{mol/litre}$)	Clearance ($\mu\text{l/h}$ per mg of cell protein)	Degradation (% of endo- cytosed amount)	Adsorption (nmol of GlcA/mg of cell protein)
0	49.7	95	0.7
0.23	53.8	94	4.7
2.3	27.1	92	4.4
23	15	71	8.6

effect was caused by a diminished charge repulsion between proteoglycans and the cell surface. In a control experiment endocytosis of β -*N*-acetylhexosaminidase from fibroblast secretions by Sandhoff fibroblasts was measured. Even in the presence of $23\ \mu\text{mol}$ of poly-L-lysine/litre the enzyme was internalized at the same rate as in untreated cultures (results not shown).

Finally, fibroblasts were exposed for 2 h to 3 mM-*S*-2-aminoethylcysteine, a lysine analogue, in lysine-free Waymouth medium and then labelled with $^{35}\text{SO}_4^{2-}$ in the continued presence of the analogue for 18 h. This treatment decreased the sulphate incorporation into secreted proteoglycans by 64% when compared with a control culture incubation without the analogue. Similar doses of native and modified proteoglycans were added to fibroblast cultures. Compared with the clearance of

native proteoglycans (59.8 μ l/h per mg of protein), the uptake of proteoglycans secreted in the presence of the analogue was reduced to 44%. The quantity that became associated with the cell membrane was also decreased (43% of control), whereas the degradation of the glycosaminoglycan chains proceeded normally.

Taking all results together, we propose that the recognition marker for receptor-mediated endocytosis of proteodermatan sulphate by cultured skin fibroblasts resides in the protein moiety of these macromolecules for the following reasons. (i) The presence of dermatan sulphate chains and of *N*-glycosidically linked oligosaccharides is not required to allow efficient uptake; the results of competition experiments did not suggest a role of *O*-glycosidically linked oligosaccharides. (ii) Chemical or biosynthetic modification of lysine residues causes a decrease in the uptake rate. (iii) Reversal of lysine modification reverses the effect on endocytosis. The latter findings are similar to those on the clearance mechanism of low-density lipoproteins (Mahley *et al.*, 1980). Previous studies have shown that limited tryptic digestion of core proteins also leads to a marked decrease in the amount of endocytosable material (Truppe & Kresse, 1978). The precise structural features of the recognition marker, however, are still unknown.

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