Biochem. J. (1989) 263, 137–142 (Printed in Great Britain)

Endocytosis of a small dermatan sulphate proteoglycan

Identification of binding proteins

Heinz HAUSSER, Willi HOPPE,* Uwe RAUCH and Hans KRESSE†
Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, D-4400 Münster, Federal Republic of Germany

Endosomal preparations from human osteosarcoma cells and from fibroblasts contain 51000- and 26000-M_r proteins which bind a small dermatan sulphate proteoglycan after SDS/polyacrylamide-gel electrophoresis and Western blotting. Binding can be inhibited by unlabelled proteoglycan core protein. The proteins co-precipitate with a proteoglycan core protein–antibody complex. Scatchard analysis of immobilized endosomal proteins yielded a K_D of about 37 nM for the proteoglycan. In intact cells proteins of the same size can be found. They are sensitive to trypsinization. A 51000-M_r protein is the predominant membrane protein with strong binding to immobilized dermatan sulphate proteoglycan. There are additional proteoglycan-binding proteins with M_r values of around 30000 and 14000 which are insensitive to trypsin treatment. In contrast with the 51000- and 26000-M_r proteins, they resist deoxycholate/Triton X-100 extraction several days after subcultivation.

INTRODUCTION

The small dermatan sulphate proteoglycan (DS-PG II) from human fibroblast secretions is one of two different interstitial small proteoglycans (Rosenberg *et al.*, 1985; Heinegård *et al.*, 1985) and has been found in all tissues so far investigated (Voss *et al.*, 1986). The mature form consists of a core protein with an M_r of 36319 as deduced from cloned cDNA (Krusius & Ruoslahti, 1986), a single glycosaminoglycan chain being linked to the serine residue at position 4 (Chopra *et al.*, 1985) and either two or three asparagine-bound oligosaccharides (Glössl *et al.*, 1984).

Small DS-PG II has been shown to be located at the 'd' band of type I collagen fibrils (see Scott, 1988 and references therein). Neither the 17-amino acid *N*-terminal peptide nor the glycosaminoglycan chain was required for interaction with a collagen matrix (Vogel *et al.*, 1987). Binding of DS-PG II to fibronectin fibrils similarly involves a protein–protein interaction (Schmidt *et al.*, 1987). Immunogold labelling of DS-PG II on the surface of cultured fibroblasts reveals, in addition to a fibrillar labelling pattern, an arrangement of the antigen in clusters. These clusters are considered to represent small DS-PG II in the process of endocytosis (Völker *et al.*, 1985).

Endocytosis of DS-PG II by a variety of cultured cells is a highly efficient, specific and saturable process, as has been proposed for receptor-mediated endocytosis (Truppe & Kresse, 1978; Prinz *et al.*, 1978). The recognition marker for uptake which is very sensitive to denaturing conditions resides in the protein moiety of the proteoglycan and involves lysine residues (Glössl *et al.*, 1983). The receptor required for core protein binding and subsequent endocytosis, however, has not yet been identified. In this report we present the results of binding studies which suggest that 51000- and 26000-M_r proteins are involved in the uptake of DS-PG II.

EXPERIMENTAL

Materials

The human osteosarcoma cell line MG-63 was obtained from the American Type Culture Collection (Bethesda, MD, U.S.A.). The following materials were purchased from the suppliers indicated: sodium [^{35}S]sulphate (carrier-free) and [^{35}S]methionine (sp. radioactivity 30 TBq/mmol; Amersham-Buchler, Braunschweig, Germany), chondroitin ABC lyase (EC 4.2.2.4; Seikagaku Kogyo, Tokyo, Japan), nitrocellulose membranes BA 83 (0.2 μm; Schleicher & Schuell, Dassel, Germany), Zwittergent 3-12 (Calbiochem, Frankfurt, Germany), Triton X-100 (Merck, Darmstadt, Germany), sodium deoxycholate, pepstatin A and leupeptin (Sigma, Deisenhofen, Germany). The Bio-Gel TSK DEAE-5 PW column (7.5 mm × 75 mm) was from Bio-Rad, München, Germany and AH-Sepharose 4B from Pharmacia LKB G.m.b.H., Freiburg, Germany.

Buffers

Buffer A consisted of 20 mM-Tris/HCl, pH 7.4. Buffer B contained 10 mM-triethanolamine, pH 7.4, 10 mM-EDTA, 5 mM-benzamidine, 10 mM-*N*-ethylmaleimide, 2 μM-leupeptin and 1.5 μM-pepstatin A. Buffer C was composed of 20 mM-Tris/HCl, pH 7.4, 0.15 M-NaCl, 0.1 M-6-aminohexanoic acid, 10 mM-EDTA, 5 mM-benzamidine, 10 mM-*N*-ethylmaleimide, 0.5% (w/v) sodium deoxycholate and 0.5% (v/v) Triton X-100. Buffer D contained 18 mM-sodium phosphate, pH 7.4, 137 mM-NaCl, 3 mM-KCl and protease inhibitors as in buffer C.

Abbreviation used: DS-PG II, small dermatan sulphate proteoglycan from fibroblast secretions.

* Present address: Department of Medical Chemistry, University of Osnabrück, Osnabrück, Federal Republic of Germany.
† Correspondence address: Institute of Physiological Chemistry and Pathobiochemistry, Waldeyerstr. 15, D-4400 Münster, Federal Republic of Germany.

Preparation of DS-PG II and its core protein

DS-PG II was prepared from the secretions of human skin fibroblasts grown from healthy individuals and maintained in culture as described (Cantz *et al.*, 1972). Conditioned media were subjected to an ammonium sulphate precipitation step (Glössl *et al.*, 1984). The precipitate was dissolved in 1 ml of buffer A/0.15 M-NaCl per 20 ml of original medium and applied at a flow rate of 0.5 ml/min to a TSK DEAE-5 PW Bio-Gel column. The column was then operated at a flow rate of 1.2 ml/min. After washing with starting buffer (up to $A_{210} < 0.05$), a gradient of NaCl in buffer A was applied as follows: 0.15–0.35 M-NaCl (10 ml), 0.35 M-NaCl (19 ml), 0.35–0.58 M-NaCl (1 ml), 0.58–0.68 M-NaCl (5 ml). DS-PG II was eluted at the step from 0.35 M to 0.58 M-NaCl, whereas about 90% of total heparan sulphate proteoglycans were eluted at lower NaCl concentrations. [^{35}S]Sulphate-labelled DS-PG II was prepared analogously after incubation of fibroblasts in the presence of 0.37 MBq of [^{35}S]sulphate/ml for 72 h. Streptomycin sesquisulphate was omitted, and fetal calf serum had been dialysed against 0.15 M-NaCl.

DS-PG II core protein was prepared for competition experiments after dialysis of the proteoglycan against 20 mM-NaCl and digestion of the 4-fold concentrated solution with 2 munits of chondroitin ABC lyase/ml of starting medium in the presence of protease inhibitors (Glössl *et al.*, 1984) for 20 h at 37 °C. The enzyme was inactivated by heating to 50 °C for 5 min.

Preparation of endosomes

Endosomes were prepared from MG-63 cells and occasionally from fibroblasts. Cells from a growth area of 30 cm^2 were scraped into 1 ml of buffer B/0.25 M-sucrose. After 20 strokes with a tight-fitting Dounce homogenizer, the suspension was subjected to differential centrifugation (Evans, 1985). Appropriate fractions were layered on top of a sucrose (w/w) gradient made with 1 ml of 56%, 5 ml of 37%, 7.5 ml of 33% and 5 ml of 23% sucrose, all in buffer B, and centrifuged (Evans, 1985). Endosomes are found in the 23% sucrose layer. When MG-63 cells were challenged with [^{35}S]sulphate-labelled DS-PG II at 21 °C, 20% of the radioactivity found in the post-nuclear supernatant appeared in this fraction. At 21 °C, almost no fusion between endosomes and lysosomes occurs (Diaz *et al.*, 1988). The endosomal preparation was diluted to 0.25 M-sucrose, pelleted and washed with 20 mM-Tris/HCl, pH 7.4/0.15 M-NaCl and the protease inhibitors of buffer B.

Binding studies

Monolayer cultures were extracted for 15 min at 4 °C with 2 ml of buffer C per 75 cm^2 growth area. After centrifugation for 5 min at 10000 *g*, the extract was dried under reduced pressure, washed with methanol and subjected to SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970, as modified by Hasilik & Neufeld, 1980) followed by Western blotting (Svoboda *et al.*, 1985). Endosomes were dissolved in sample buffer and treated similarly. After blotting, the nitrocellulose membrane was rinsed with buffer A/0.15 M-NaCl and treated overnight at 4 °C with 3% bovine serum albumin in this buffer. The blocking buffer was then replaced by a fresh solution containing additionally 1×10^5–5×10^5 c.p.m. of [^{35}S]sulphate-labelled DS-PG II/ml, and binding was allowed to occur over a period of 90 min at ambient temperature. The membrane was washed seven times for 5 min each with buffer A/0.15 M-NaCl, dried and exposed for autoradiography to a preflashed Kodak X-OMAT AR film. Scanning of the film was performed with a model 2202 laser densitometer (LKB, Bromma, Sweden). For Scatchard analysis, endosomal proteins were dissolved in buffer C and spotted on to nitrocellulose membranes prior to analogous treatments with blocking solution, ligand and washing buffer. Bound and unbound radioactivity were determined by liquid scintillation counting.

For affinity chromatography, dermatan sulphate (18 mg) and DS-PG II (7 mg) were coupled to AH-Sepharose 4B (3 ml) according to the instructions of the manufacturer. Fibroblasts were incubated for 5 h in the presence of 1.1 MBq of [^{35}S]methionine/ml in methionine-free Waymouth MAB 87/3 medium (Glössl *et al.*, 1984). At the end of the incubation, surface-bound DS-PG II was removed at least in part by exposing the cells for 15 s to 0.1 M-sodium acetate/0.05 M-NaCl, pH 4.0. The cells were suspended in buffer D and homogenized as described above. From the post-nuclear supernatant, a crude membrane fraction was obtained by centrifugation for 30 min at 110000 *g* and dissolved in buffer D which contained additionally 0.5% (w/v) Zwittergent 3-12. The solution was passed over the dermatan sulphate affinity column equilibrated with the same buffer. Unbound material was applied to the DS-PG II affinity column which was operated as described in the legend of Fig. 5.

Other methods

Endocytosis of DS-PG II was followed exactly as described (Glössl *et al.*, 1983). Hexuronic acids (Bitter & Muir, 1962) and protein (Lowry *et al.*, 1951) were quantified as stated previously. Silver staining of polyacrylamide gels was performed according to Merril *et al.* (1981).

RESULTS AND DISCUSSION

Binding of DS-PG II by endosomal proteins

As stated above, DS-PG II interacts with the endocytosis receptor, and also with several extracellular matrix proteins which are associated with the cell membrane of cultured mesenchymal cells. In an attempt to identify binding proteins involved in endocytosis, an endosomal fraction was therefore prepared from fibroblasts and from osteosarcoma cells. MG-63 osteosarcoma cells internalize DS-PG II by receptor-mediated endocytosis at a similar rate to skin fibroblasts. When fibroblasts and MG-63 cells were challenged with 60000 c.p.m. of [^{35}S]sulphate-labelled DS-PG II/ml for 6 h, clearance rates of 26 and 22 μl/h per mg of cell protein respectively were calculated for the two cell types. Endosomal preparations were subjected to SDS/polyacrylamide gel electrophoresis and Western blotting. It is shown in Fig. 1 that in MG-63 endosomes [^{35}S]sulphate-labelled DS-PG II was bound by proteins of M_r 51000 and 26000. Binding was suppressed by 75% in the presence of a 40-fold excess of unlabelled DS-PG II core protein which had been heat-treated to inactivate chondroitin ABC lyase. Bound ligands could be removed in part by washing the blot with 0.35 M-NaCl in buffer A. An almost

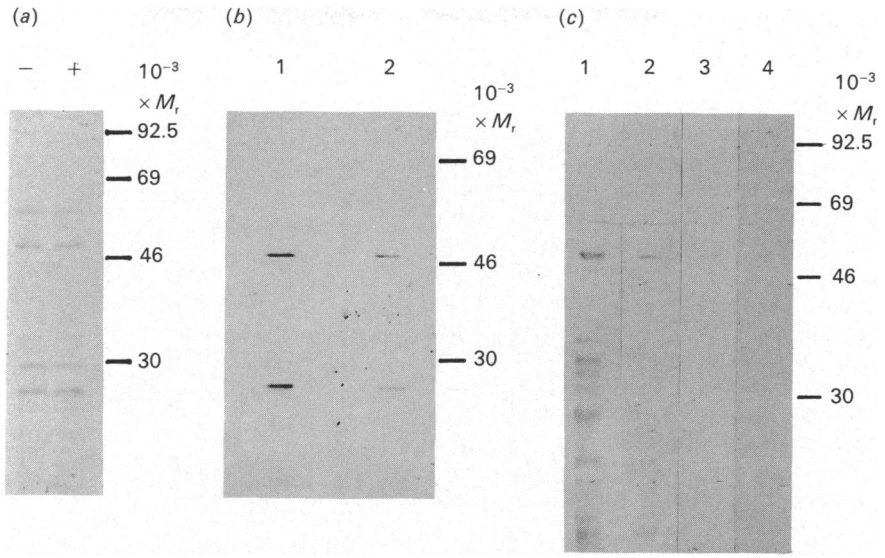

Fig. 1. DS-PG II binding proteins in endosomes from human skin fibroblasts (*a*) and osteosarcoma cells (*b* and *c*)

Endosomal proteins (about 12 μg/track in *a* and 70 μg/track in *b* and *c*) were separated by SDS/polyacrylamide-gel electrophoresis. The acrylamide concentration of the separation gel was 12.5%. Endosomal proteins were electrophoresed before (−) and after (+) reduction with 10 mM-dithiothreitol. Reduction was omitted in *b* and *c*. After blotting, 250000 c.p.m./ml (*a* and *c*) or 125000 c.p.m./ml (*b*) of [^{35}S]sulphate-labelled DS-PG II (35000 c.p.m./nmol of hexuronic acid) were added as ligand. In (*b*), core protein equivalent to 160 nmol of hexuronic acid/ml was added to the track 2 incubation, whereas the incubation solution for track 1 contained the buffer used during core protein preparation plus heat-inactivated chondroitin ABC lyase. After blotting and incubation with ligand as described in the Experimental section, the tracks in (*c*) were washed twice, for 1 min each, with buffer A/0.15 M-NaCl and then five times, for 5 min each, with buffer A/0.05 M-NaCl (track 1), buffer A/0.15 M-NaCl (track 2), buffer A/0.35 M-NaCl (track 3), or 0.05 M-sodium acetate buffer, pH 4.0, containing 0.1 M-NaCl (track 4). All washings were performed at ambient temperature. The following [^{14}C]methylated M_r standards were used: phosphorylase *b* (92500), bovine serum albumin (69000), ovalbumin (46000) and carbonic anhydrase (30000).

complete removal occurred on exposure to 50 mM-sodium acetate buffer, pH 4.0, containing 0.1 M-NaCl (Fig. 1*c*). Endosomal preparations from fibroblasts contained additional binding proteins of M_r 60000 and 30000 that were barely detectable in MG-63 endosomes (Fig. 1*a*). Treatment with dithiothreitol affected neither the electrophoretic mobility of the binding proteins nor their binding properties. Addition of either 2 mM-$CaCl_2$ or 2 mM-EGTA to the ligand solution did not influence the binding of the proteoglycan (results not shown).

The existence of several binding proteins could indicate that the radioactive probe used for Western blotting contained more than one ligand. As shown previously (Rauch *et al.*, 1986), secreted proteoglycans from fibroblasts contain about 85% DS-PG II, 10% heparan sulphate proteoglycans and 5% large chondroitin sulphate/dermatan sulphate proteoglycans. About 90% of total [^{35}S]sulphate-labelled heparan sulphate was removed during DS-PG II purification. Additional evidence that ligand binding was not due to the presence of heparan sulphate was obtained as follows. The ligand was treated for 2 h at 37 °C with chondroitin ABC lyase or buffer alone and dialysed against buffer A/0.15 M-NaCl. The enzyme-treated sample contained 21% of the radioactivity of the control sample (a longer incubation was avoided because of potential core protein denaturation), and binding by the 51000- and 26000-M_r proteins from MG-63 cells was reduced by 83 and 90% respectively. The direct approach of studying the interaction of heparitinase-digested proteoglycans with endosomal proteins has not been performed because of the contamination of the enzyme preparations with pro-

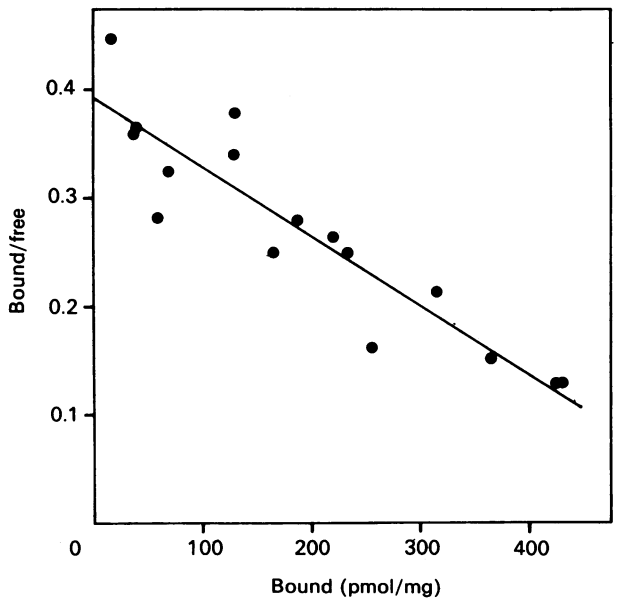

Fig. 2. Scatchard analysis of DS-PG II binding to endosomal proteins from MG-63 cells

Endosomal proteins (19 μg per assay) were immobilized on nitrocellulose membranes and, after treatment with blocking solution, were incubated in a total volume of 790 μl with various amounts of [^{35}S]sulphate-labelled DS-PG II (5.5×10^6 c.p.m./nmol of DS-PG II). The molarity of DS-PG II was calculated on the basis of 62 hexuronic acid residues per molecule.

teases that in our hands could not be inhibited without affecting the degradation of heparan sulphate.

It was not possible to separate large chondroitin sulphate/dermatan sulphate proteoglycans from DS-PG II by non-denaturing chromatographic methods. However, a Scatchard analysis of endosomal proteins showed that 30% of the radioactivity of the ligand solution could be bound (Fig. 2). Thus, although the contribution of large proteoglycans to total binding cannot be rigorously excluded, the sample contains only about 6–7% of proteoglycans other than DS-PG II. Therefore, even if the other proteoglycans were bound completely to the endosomal proteins, an interaction between DS-PG II and these proteins accounts for at least 80% of bound radioactivity. It should also be noted that large chondroitin sulphate/dermatan sulphate proteoglycans are not taken up by receptor-mediated endocytosis in a variety of mesenchymal cells (von Figura *et al.*, 1980; Schmidt & Buddecke, 1985).

Evidence for a specific interaction of DS-PG II core protein with 51000- and 26000-M_r proteins was also obtained by incubating 0.5 μg of DS-PG II core protein with endosomal proteins followed by an immune reaction with a monospecific antibody against the core protein (Fig. 3). The incubation was performed under less stringent conditions than used for immune precipitation of cell extracts (0.15 M-NaCl instead of 1.0 M-NaCl; inclusion of 0.5% Triton X-100 and 0.5% sodium deoxycholate in the washing buffers only). Silver staining of the electrophoretogram revealed that under these conditions 51000- and 26000-M_r proteins were reproducibly found in the immune precipitate. Much smaller amounts of these proteins were found when the exogenous addition of core protein was omitted from the incubation mixture. Minor amounts of 51000- and 26000-M_r proteins were also seen after treatment of endosomes and core protein with control serum. We found, however, that all our control sera reacted with DS-PG II core protein, the titre being 2000-fold lower than that of the antiserum in an e.l.i.s.a. test system (Schmidt *et al.*, 1987). Without serum, the 51000- and 26000-M_r proteins were barely if at all detectable. DS-PG II binds to the 51000- and 26000-M_r proteins apparently with a similar affinity, as shown by Scatchard analysis (Fig. 2). Maximally, 620 pmol of DS-PG II was bound by 1 mg of endosomal proteins. Non-specific binding was less than 5%, as judged from the binding in the presence of excess amounts of unlabelled DS-PG II (Fig. 4). The dissociation constant, K_D, of 37 nM was one order of magnitude higher than that of the asialoglycoprotein receptor (Schwartz *et al.*, 1981), the low-density lipoprotein receptor (Goldstein *et al.*, 1976) or the transferrin receptor (Ciechanover *et al.*, 1983). In the latter cases, however, more physiological conditions could be used for measurements of the dissociation constant.

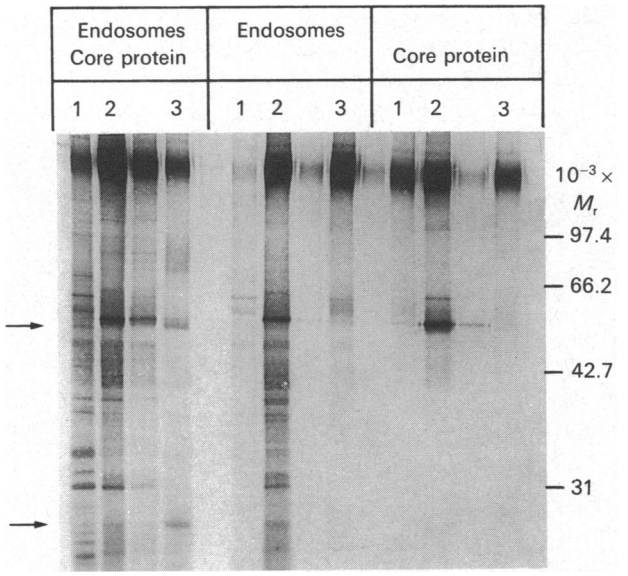

Fig. 3. Immune precipitation of DS-PG II binding proteins

Endosomal proteins (18 μg in 95 μl) from MG-63 cells in buffer A containing 0.15 M-NaCl, the protease inhibitors of buffer B and 0.05% Zwittergent 3-12 were mixed with 5 μl of core protein (0.55 μg) or buffer and incubated for 15 h at 4 °C. The incubation mixture was then sequentially treated with Protein A–Sepharose preadsorbed with control serum and antiserum respectively, and subjected to SDS/polyacrylamide-gel electrophoresis under non-reducing conditions prior to silver staining. Lane 1, control serum precipitate; lane 2, supernatant after antiserum treatment; lane 3, immune precipitate. M_r standards were the unlabelled proteins listed in Fig. 1. The arrows indicate the mobility of 51000- and 26000-M_r proteins. There is some overflow from the heavily loaded lanes 2 to the neighbouring lanes.

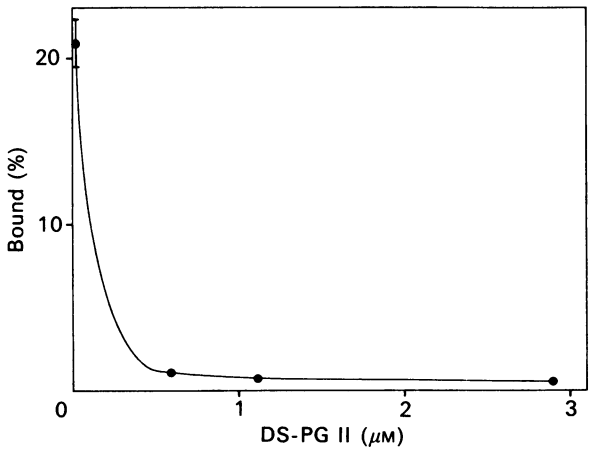

Fig. 4. Binding of DS-PG II to endosomal proteins from MG-63 cells

Binding conditions were as described in the legend to Fig. 2, except that various doses of unlabelled DS-PG II were added to a 20 nM solution of labelled ligand; the radioactivity was 110000 c.p.m./ml. If not otherwise indicated, the results of duplicate determinations fell within the range of symbols.

Binding of DS-PG II by whole cell extracts

Endocytosis receptor proteins are expected to shuttle between the plasma membrane and endosomal compartments. To investigate the binding of biosynthetically labelled receptor proteins to DS-PG II, a membrane fraction of [^{35}S]methionine-labelled fibroblasts was therefore prepared and chromatographed first on a dermatan sulphate affinity column to remove cationic proteins and then on a DS-PG II affinity column. The ligands had been coupled via their carboxy groups because of the

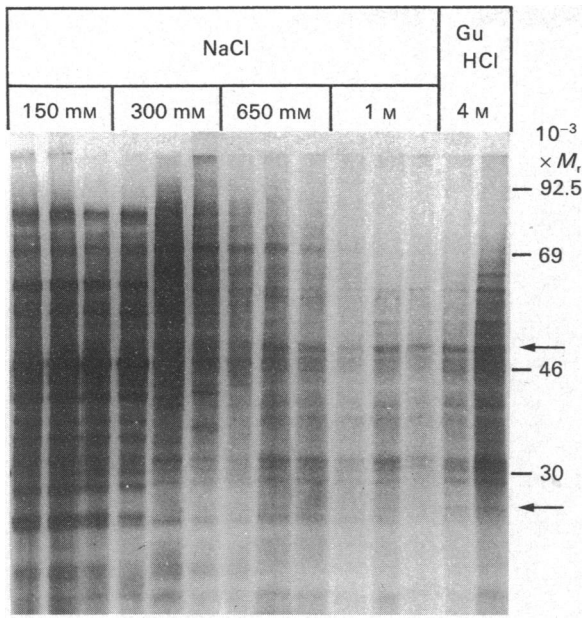

Fig. 5. Binding of [^{35}S]methionine-labelled membrane proteins to immobilized DS-PG II

[^{35}S]Methionine-labelled membrane proteins were obtained from fibroblasts from a growth area of 150 cm^2. Material which was not retained by the dermatan sulphate affinity column was loaded on a 2 ml DS-PG II affinity column being equilibrated with buffer D/0.5% Zwittergent 3-12. The column was eluted stepwise in 0.5 ml fractions with starting buffer (3 ml) and then with this buffer with NaCl concentrations of 0.3 M (2.5 ml), 0.65 M (2.5 ml) and 1 M (4 ml). Final desorption was performed with 2.5 ml of buffered 4 M-guanidinium hydrochloride (Gu HCl). Each third, fourth and fifth fraction was made 0.1% with SDS and dialysed against water prior to SDS/polyacrylamide-gel electrophoresis and fluorography. Only aliquots of the breakthrough fractions were applied. Of the applied radioactivity, 6% was retained by the column. The arrows indicate the mobility of 51000- and 26000-M_r proteins.

known involvement of lysine residues of the core protein in receptor binding (Glössl *et al.*, 1983). Several proteins which were not retained by the protein-free dermatan sulphate affinity column interacted strongly with the DS-PG II column (Fig. 5). The protein fractions desorbed in the presence of 1 M-NaCl or 4 M-guanidinium hydrochloride showed major bands with M_r values of 51000 and of 29000–32000. A discrete band of a 26000-M_r protein was desorbed under the same conditions as the 51000-M_r protein. The 29000–32000-M_r proteins could also be detected by Western blotting of total cell extracts (see below). The existence of several other minor protein contaminants remains unexplained at present, but it should be noted that bound radioactivity rechromatographed with similar characteristics.

In a final set of experiments, intact fibroblasts were extracted with detergents in the presence of protease inhibitors and again subjected to SDS/polyacrylamide-gel electrophoresis and Western blotting. Freshly trypsinized cells were devoid of 51000-M_r binding protein, but the protein reappeared after a longer period of time (Fig. 6*a*). The 26000-M_r protein was weakly detectable, again after a long subculturing period only. On the contrary, binding proteins of 30000- and 31000-M_r and of 13000- and 14000-M_r were detectable immediately after trypsinization. These proteins were barely extractable with Triton X-100 and deoxycholate in confluent cultures, i.e. four or more days after subculturing. They were regularly seen, however, when the extraction was performed with 1% SDS (Fig. 6*b*). No binding was observed when the same extract as used in Fig. 6(*b*) was incubated with the glycosaminoglycans obtained by β-elimination from the DS-PG II preparation used as ligand.

The multitude of DS-PG II binding proteins in fibroblasts and osteosarcoma cells which have been identified in this study was quite unexpected. Quenching experiments with core protein and incubation with glycosaminoglycans suggested that in all cases the interaction was dependent on the presence of the core protein. Several independent experiments gave rise to the conclusion that the 51000- and 26000-M_r proteins are involved in the endocytotic uptake of DS-PG II. First, they were found in endosomes by Western blotting. Secondly, binding could be reversed at pH 4.0. Thirdly, the proteins could be immune-precipitated from solubilized endosomes by antibodies against the core protein if core protein was added as ligand. Fourthly, they disappeared after trypsin treatment of intact cells. Due to the rapid recycling of endocytosis receptors between the plasma membrane and internal compartments (see Thilo, 1985 and Wileman *et al.*, 1985 for reviews), proteolytic degradation of the majority of receptor proteins could occur during the 15 min of trypsinization. Once these proteins reappeared they remained extractable. Such a behaviour has to be postulated for a recycling receptor.

The relationship between the 51000- and 26000-M_r proteins is not known at present. The smaller species could have been derived from the larger one by proteolysis during the preparation of the endosomes. It has not been established whether sufficient amounts of protease inhibitors are able to penetrate the endosomal membrane. Proteolysis of endocytosed proteins is known to begin within prelysosomal compartments (Schaudies *et al.*, 1987). The possibility that there is a precursor–product relationship between these proteins is supported by the observation that the 26000-M_r protein was barely detectable in whole cell extracts but became more prominent if protease inhibitors were omitted from the extraction buffer (Fig. 6*c*).

The stoichiometry of the interaction of DS-PG II and the binding proteins remains to be determined. Protein staining after immune precipitation of endosomal proteins in the presence of core protein suggests that an excess of binding proteins co-precipitates with the core protein (Fig. 3). The core protein contains seven repetitive sequences (Krusius & Ruoslahti, 1986), several of which contain conserved lysine residues. It seems possible, therefore, that the core protein interacts simultaneously with more than one protein, but self-association of binding proteins must also be considered.

The localization and function of those binding proteins that required SDS for extraction from confluent cultures are not known at present. As stated above, DS-PG II interacts with several components of the extracellular matrix, and its core protein could bind to lectins because of the presence of asparagine-bound oligosaccharides (Glössl *et al.*, 1984). In this connection it should also be noted that there are profound differences in the proteo-

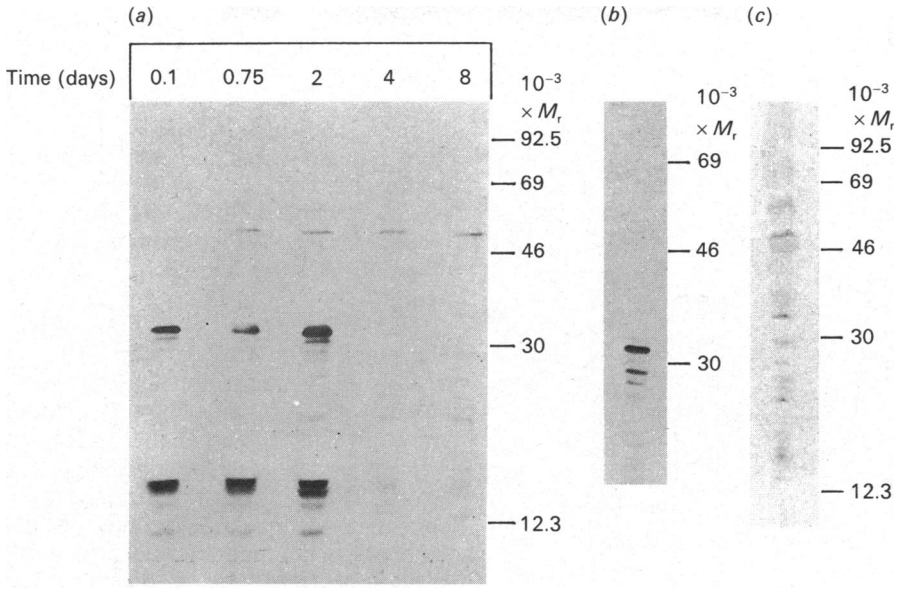

Fig. 6. Binding of DS-PG II to fibroblast extracts

(*a*) Trypsinized fibroblasts were replated after 3-fold dilution and harvested at the time indicated as described in the Experimental section. Protein (40 μg) was applied to each lane prior to electrophoresis and Western blotting. (*b*) 10 days after subcultivation, fibroblasts were extracted with the sample buffer used for SDS/polyacrylamide-gel electrophoresis. (*c*) Confluent fibroblasts (6 days after subculturing) were treated as in (*a*), but protease inhibitors were not used during extraction.

glycan distribution pattern between MG-63 cells and fibroblasts; the former produce only small amounts of DS-PG II (E. Korsching & H. Kresse, unpublished work). It remains to be investigated whether the 60000- and 30000-M_r proteins which have been found in the endosomes from fibroblasts are related to the predominance of DS-PG II in this cell type.

We are indebted to D. Holtfrerich and L. Niestert for their skilled technical assistance. This work was supported in part by the Deutsche Forschungsgemeinschaft (SFB 310) and by the Braun-Stiftung.

REFERENCES

Bitter, T. & Muir, H. M. (1962) Anal. Biochem. **4**, 330–334
Cantz, M., Kresse, H., Barton, R. W. & Neufeld, E. F. (1972) Methods Enzymol. **28**, 884–897
Chopra, R. K., Pearson, C. H., Pringle, G. A., Fackre, D. S. & Scott, P. G. (1985) Biochem. J. **232**, 277–279
Ciechanover, A., Schwartz, A. L. & Lodish, H. F. (1983) Cell **32**, 267–275
Diaz, R., Mayorga, L. & Stahl, P. (1988) J. Biol. Chem. **263**, 6093–6100
Evans, W. H. (1985) Methods Enzymol. **109**, 246–257
Glössl, J., Schubert-Prinz, R., Gregory, J. D., Damle, S. P., von Figura, K. & Kresse, H. (1983) Biochem. J. **215**, 295–301
Glössl, J., Beck, M. & Kresse, H. (1984) J. Biol. Chem. **259**, 14144–14150
Goldstein, J. L., Basu, S. U., Bronstede, G. Y. & Brown, M. S. (1976) Cell **7**, 85–95
Hasilik, A. & Neufeld, E. F. (1980) J. Biol. Chem. **255**, 4937–4945
Heinegård, D., Björne-Persson, A., Cöster, L., Franzén, A., Gardell, S., Malmström, A., Paulsson, M., Sandfalk, R. & Vogel, K. (1985) Biochem. J. **230**, 181–194
Krusius, T. & Ruoslahti, E. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7683–7687
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Merril, C. R., Goldman, D. & Ebert, M. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 6471–6475
Prinz, R., Schwermann, J., Buddecke, E. & von Figura, K. (1978) Biochem. J. **176**, 671–676
Rauch, U., Glössl, J. & Kresse, H. (1986) Biochem. J. **238**, 465–474
Rosenberg, L. C., Choi, H. U., Tang, L.-H., Johnson, T. L., Pal, S., Webber, C., Reiner, A. & Poole, A. R. (1985) J. Biol. Chem. **260**, 6304–6313
Schaudies, R. P., Gorman, R. M., Savage, C. R., Jr. & Poretz, R. D. (1987) Biochem. Biophys. Res. Commun. **143**, 710–715
Schmidt, A. & Buddecke, E. (1985) Eur. J. Biochem. **153**, 269–273
Schmidt, G., Robenek, H., Harrach, B., Glössl, J., Nolte, V., Hörmann, H., Richter, H. & Kresse, H. (1987) J. Cell Biol. **104**, 1683–1691
Schwartz, A. L., Fridovich, S. E., Knowles, B. B. & Lodish, H. F. (1981) J. Biol. Chem. **256**, 8878–8881
Scott, J. E. (1988) Biochem. J. **252**, 313–323
Svoboda, M., Meuris, S., Robyn, C. & Christophe, J. (1985) Anal. Biochem. **151**, 16–23
Thilo, L. (1985) Biochim. Biophys. Acta **822**, 243–266
Truppe, W. & Kresse, H. (1978) Eur. J. Biochem. **85**, 351–356
Vogel, K. G., Koob, T. J. & Fisher, L. W. (1987) Biochem. Biophys. Res. Commun. **148**, 658–663
Völker, W., Schmidt, A., Buddecke, E., Themann, H. & Robenek, H. (1985) Eur. J. Cell Biol. **36**, 58–65
von Figura, K., Mittelviefhaus, H., Prinz, R., Duchene, M. & Krieg, T. (1980) in Biology of the Articular Cartilage in Health and Disease (Gastpar, H., ed.), pp. 189–195, F. K. Schattauer, Stuttgart and New York
Voss, B., Glössl, J., Cully, Z. & Kresse, H. (1986) J. Histochem. Cytochem. **34**, 1013–1019
Wileman, T., Harding, C. & Stahl, P. (1985) Biochem. J. **232**, 1–14

Received 13 February 1989/17 April 1989; accepted 4 May 1989