Modulation of collagen gel contraction by decorin

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The small dermatan sulphate protein decorin interacts via its core protein with fibrillar collagens, and its glycosaminoglycan chains were proposed to be capable of self-association. It was therefore of interest to study the role of decorin in the contraction of cell-populated collagen lattices. Stable transfection of dihydrofolate reductase-deficient CHO cells with decorin cDNA resulted in impaired collagen lattice contraction. Using normal human skin fibroblasts in serum-free cultures, inclusion of 0.3 μM decorin in the culture medium also led to a delayed collagen gel contraction. Protein-free dermatan sulphate and the dermatan sulphate-degrading enzyme chondroitin ABC lyase were ineffective. Potential interactions between dermatan sulphate chains were studied by gel filtration. A shift in the elution position of [^{35}S]sulphate-labelled decorin-derived glycosaminoglycans by unlabelled decorin could be observed only when the chains were prepared by trypsin. Chains liberated by β-elimination or by cathepsin C were eluted at identical positions in the presence or absence of decorin. It is therefore unlikely, that the effect of decorin on collagen-gel retraction is brought about solely by glycosaminoglycan–glycosaminoglycan interactions.

INTRODUCTION

Mesenchymal cells such as fibroblasts are surrounded *in vivo*, but not in monolayer culture, by a fibrous extracellular matrix which exerts a profound influence on gene expression and on shape and behaviour of the cells. Bell et al. [1] have therefore developed an *in vitro* culture system in which fibroblasts are embedded in a three-dimensional collagen lattice. Entrapment of cells within the hydrated collagen gel results in the generation of contractile forces which are transmitted throughout the matrix along continuously interconnected collagen fibrils [2] and which lead to contraction of the collagen gel. Gel contraction results in a dermal-like tissue, and hence fibroblast-populated collagen lattices represent a useful *in vitro* model for studying various aspects of wound healing and connective tissue regeneration.

Previous studies have shown that collagen-gel contraction depends on the type [3] and number [1,4] of cells and on an intact cytoskeleton [5]. Collagen-gel contraction by fibroblasts requires cellular fibronectin but not plasma fibronectin [6]. However, contraction is not dependent on $\alpha5\beta1$ integrin [7], a high-affinity fibronectin receptor, but requires $\alpha2\beta1$ integrin, a collagen receptor [8,9]. The other collagen receptor, $\alpha1\beta1$ integrin, does not seem to be involved in collagen lattice contraction. Contraction-stimulating activities have been attributed to transforming growth factor β (TGF-β) [10,11], some isoforms of the plateled-derived growth factor [12,13] and endothelins [14]. In contrast, interleukin 1 inhibited initial collagen-gel retraction and induced degradation of the gel at later stages [11,15].

In the tissue as well as in fibroblast-populated collagen gels, collagen fibrils become decorated with a small dermatan sulphate protein which was named decorin because of this property [16–20]. Triple-helical type-I collagen possesses a specific decorin-core-protein-binding site at the d-band in each D period [16,18,21]. As decorin binds to the surface of collagen fibrils, the lateral assembly of triple-helical molecules is delayed [22] and the fibrils become thinner [23]. Decorin carries a single chondroitin/dermatan sulphate chain only. Self-association of dermatan sulphate has been reported to occur [24,25]. This property formed the basis of the proposal that, at least in certain tissues, parallel oriented collagen fibrils are bridged by laterally and longitudinally aggregated dermatan sulphate chains [26], thereby attributing to decorin a specific role in establishing a collagen-banding pattern. On the basis of the model of collagen–decorin and decorin–decorin interactions, it seemed attractive to assume an influence of decorin on the contraction of fibroblast-populated collagen lattices. We therefore investigated the effect of decorin overexpression, the effects of exogenously added decorin and the consequences of enzymic removal of dermatan sulphate chains on the kinetics of collagen-gel retraction. The results of this study are presented here.

EXPERIMENTAL

Cell culture

Skin fibroblasts from healthy donors (3–15 years of age, 4th to 15th passage) and from a patient with a defect in xylosylprotein β-galactosyltransferase [27] were cultured in modified and supplemented Eagle's minimum essential medium with Earle's salts as described [28]. Fibroblast-populated floating collagen lattices were prepared in hydrophobic Petri dishes (Greiner, Nürtingen, Germany; article number 627102) using acid-soluble type-I collagen from calf skin (Sigma, Deisenhofen, Germany; catalogue number C3511) by mixing the following components: 675 μl of 1.78-fold concentrated Waymouth MAB 37/3 medium (as formulated in the catalogue of Gibco, Eggenstein, Germany), 100 μl of a solution containing 150 g/l BSA (Serva, Heidelberg, Germany; catalogue number 11920), 15 μl of penicillin (1.5 units)/streptomycin (1.5 μg), 450 μl of type-I collagen (1.5 mg, dissolved in 17 mM acetic acid), 75 μl of 0.1 M NaOH, 50 μl of Waymouth medium containing the test components and 150000 fibroblasts suspended in 150 μl of Waymouth medium. Care was taken to avoid cell aggregation during the preparation of the gels by continuous gentle pipetting of the cell suspension. Chinese

Abbreviations used: CHO, Chinese hamster ovary; TGF-β, transforming growth factor β.

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hamster ovary (CHO) cells were maintained in Dulbecco's modified Eagle's medium/F12 medium (1:1, v/v) containing 5 mg of insulin/l and 10% fetal calf serum. For collagen-contraction studies, this medium was also used in place of Waymouth medium, whole fetal calf serum (10% final concentration) replaced BSA, and the gel was inoculated with 450000 cells. The cultures were maintained at 37 °C in 95% air/5% CO_2. The diameter of the gels was determined under indirect illumination by polaroid photography and measurement with a precision of 0.5 mm. In later experiments, measurements were carried out directly with a ruler since it had been ascertained that the two methods yielded the same results. All experiments were performed independently at least two or three times, and similar results were always obtained. In separate experiments there was, however, some variability in the rate of collagen-gel contraction which was dependent on the cell strain and other as yet undefined variables. Within an experimental series, parallel cultures exhibited gel diameters that differed in no case by more than 2 mm and usually by 1 mm or less.

Decorin cDNA transfection

Clone D6 which contains the coding region of human decorin core protein has been described previously [29]. Non-translatable sequences were removed by digestion with *Eco*RI and *Hpa*I, and, after treatment with Klenow fragment of DNA polymerase, the cDNA was ligated into the *Eco*RI site of the expression vector pMT2 which was propagated in *Escherichia coli* strain DH5α. The vector, kindly provided by Dr. R. Kaufman (Genetics Institute, Cambridge, MA, U.S.A.), contains a dihydrofolate reductase gene. After having verified the orientation of the insert, the vector with or without the insert was used for transfection with lipofectamine (Gibco/BRL) of DG 44 cells. These CHO cells which were generously supplied by Dr. L. Chasin (Columbia University, New York, NY, U.S.A.) are deficient in dihydrofolate reductase. Incubation of the cells in a 25 cm^2 flask with 20 μg of plasmid DNA and 40 μg of lipofectamine in 1.5 ml of serum-free Dulbecco's modified Eagle's medium/F12 medium was for 6 h at 37 °C. After selection with nucleoside-deficient α-minimal essential medium containing 10% fetal calf serum dialysed against 0.15 M NaCl, the plasmid was amplified by adding methotrexate to the culture medium up to a final concentration of 500 nM.

Preparation of proteoglycans and glycosaminoglycans

Unlabelled and [^{35}S]sulphate-labelled proteoglycans were prepared from the conditioned media of skin fibroblasts maintained under serum-free conditions as described previously [30]. Since fibronectin is often not completely removed by the procedure quoted, the samples were rechromatographed on DEAE-Trisacryl M (Serva) at pH 8.2 in the absence of protease inhibitors but under otherwise identical conditions. Since under our culture conditions fibroblasts secrete decorin as the main proteoglycan species and since heparan sulphate proteins are partially removed during purification, the final preparations contained at least 90% decorin as judged by SDS/PAGE. The quantity of decorin was calculated from the hexuronic acid content [31], 1 μmol of uronic acid being equivalent to 1.2 mg of the proteoglycan.

The dermatan sulphate chain of decorin was liberated by (i) β-elimination, (ii) trypsin or (iii) cathepsin C treatment. The β-elimination was performed by treating the lyophilized sample for 24 h at 37 °C with 0.1 M NaOH/1 M $NaBH_4$. The reaction was terminated by neutralization with 1 M acetic acid, and the sample was dialysed against water. For trypsin treatment, the proteoglycan was dissolved in 0.1 M $NaHCO_3$, pH 8.0, and mixed with 8 μl of a 2.5% trypsin solution (Boehringer, Mannheim, Germany; tissue culture grade; 150 units/ml) per 100 μl of sample. After 2 h at 37 °C the enzyme was inactivated with PMSF (1 mM final concentration) before dialysis against water. Digestion with cathepsin C was for 3 h at 37 °C with 1.8 units of enzyme (Boehringer), in 300 μl of 0.1 M sodium citrate buffer, pH 5.0. The reaction was terminated with iodoacetic acid (10 mM final concentration), followed by neutralization with 1 M Tris base and dialysis.

Digestion of collagen gels and subsequent immunoprecipitation of decorin was performed exactly as previously described [19].

Cell adhesion

To study the influence of decorin overexpression on the cellular interaction with type-I collagen, a cell adhesion assay similar to the one described by Winnemöller et al. [32] was used. Type-I collagen (Sigma, see above) was dissolved in 17 mM acetic acid (80 μg/ml), diluted with an equal volume of 36 mM sodium phosphate/0.3 M NaCl, pH 7.4, and used to coat microtitre dishes. Transfected and mock-transfected CHO cells were labelled overnight in the presence of 15 μCi/ml [^{35}S]methionine before being used for cell adhesion experiments.

Gel filtration

For studies on the interaction between glycosaminoglycan chains, decorin and [^{35}S]sulphate-labelled glycosaminoglycans were chromatographed at ambient temperature as described by Fransson [24] on a Sepharose CL-6B column (0.9 cm × 90 cm) equilibrated and eluted with 0.5 M sodium acetate buffer, pH 7.0, containing 0.05% BSA and 0.1% Triton X-100 for better recovery. Fractions of about 1.5 ml were collected at a flow rate of about 18 ml/h. For control purposes the samples were also chromatographed, under otherwise identical conditions, with a buffer containing in addition 4 M guanidinium chloride.

RESULTS

Delayed collagen-gel contraction by decorin-expressing CHO cells

CHO cells of strain DG44 expressed only trace quantities of decorin as judged by SDS/PAGE of preparations obtained from [^{35}S]sulphate-labelled secretions after purification on a DEAE-Trisacryl column (not shown). They were therefore chosen as target cells for transfection with a human decorin cDNA, the cDNA being expressed under the influence of the adenovirus major late promoter. After amplification of the plasmid, stable decorin-expressing clones were obtained. For a comparison of the quantity of decorin secreted by transfected CHO cells and fibroblasts, 10^6 cells were seeded per 25 cm^2 Falcon plastic flask, and secreted proteoglycans were purified after 2 days by anion-exchange chromatography. After chondroitin ABC lyase digestion, the samples were subjected to SDS/PAGE and Western blotting. The intensity of the immune staining of the samples was visually compared with the staining intensity of nine different standard concentrations in the range of 0.6 ng–3 μg of decorin core protein. It can be deduced from Figure 1 that fibroblasts still secreted the largest quantity of decorin (about 1.2 μg of core protein). Decorin-transfected CHO cells yielded about 300 ng of core protein in contrast with about 3 ng of core protein yielded by non-transfected CHO cells. No changes in the morphology between wild-type cells and cells transfected with decorin-cDNA-containing or decorin-free pMT2 vector were observed. This is in contrast with the findings of Yamaguchi and Ruoslahti [33], who noted that decorin expression by CHO cells is associated with an enlarged cell surface and hence a decreased cell density.

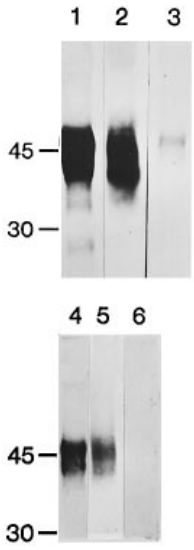

Figure 1 Decorin expression by transfected CHO cells and fibroblasts

Some 2×10^6 fibroblasts (lanes 1 and 4), decorin-transfected CHO cells (lanes 2 and 5) and control CHO cells (lanes 3 and 6) were maintained in culture for 2 days after which time the cell numbers had increased to 6×10^6 fibroblasts, 1×10^7 transfected CHO cells and 7×10^6 control CHO cells. The proteoglycans from the media were purified, digested with chondroitin ABC lyase, and subjected to SDS/PAGE at a total acrylamide concentration of 12.5% (w/v) and Western blotting. In lanes 1–3, 50% and in lanes 4–6, 5% of the obtained preparations were applied.

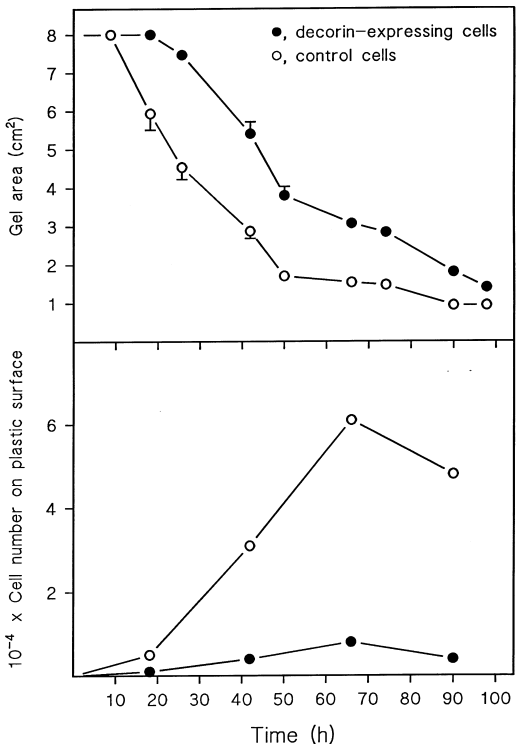

Figure 2 Effect of decorin expression on collagen-gel contraction by CHO gels

At the beginning of the experiment five parallel cultures of decorin-expressing (●) and control (○) CHO cells were established as described in the Experimental section. Each gel contained 450000 cells. The time-dependent decrease in gel surface area is shown in the upper panel. At the times indicated in the lower panel the collagen lattice was removed from one of the Petri dishes, and the number of cells that could be released by trypsin from the surface of the Petri dish was determined. The bars in the upper panel indicate the maximal deviation from the mean value of the gels that were present at the particular time point of the experiment. Bars were omitted when the deviation did not exceed the size of the symbol.

When decorin-expressing and non-expressing CHO cells were embedded in a collagen gel, contraction caused by the decorin cDNA-transfected cells was considerably delayed and the extent of final gel contraction was decreased (Figure 2, top). In one of six separate experiments, it did not even occur over a period of 3 days. When decorin-free and decorin-expressing cells were mixed in different proportions (25, 50 and 75% of one cell type), the delay in retraction correlated with the percentage of decorin-expressing cells (Figure 3).

The specificity of the different behaviour of cells expressing either large or only trace quantities of decorin was assessed as follows. No differences in cell adhesion to a type-I collagen substrate were observed. Almost complete adhesion occurred within 60 min, and the kinetics of adhesion were not different (results not shown). Spreading was not observed. Since antibodies against $\alpha 2\beta 1$ integrins of Chinese hamster were not available, a direct quantification of this collagen receptor was not possible. In spite of the rapid adherence to immobilized type-I collagen, about 25% of the 450000 CHO cells mixed with collagen at the beginning of the gel-retraction experiments did not become integrated into the floating gel when measured after 2 days of incubation. Almost no increase in cell number in the culture medium was observed afterwards. Again, no significant difference between transfected and control cells was detected. However, of the approx. 115000 cells that were not retained in the matrix in either of the cultures, more than 60000 control CHO cells but only about 8000 decorin-expressing cells were able to adhere to the hydrophobic Petri dish, the other cells remaining in suspension. This may be due to the known antiadhesive properties of decorin [32].

In a further control, decorin from fibroblast secretions (50 μg per dish) was added at the beginning of the experiment. It is evident from Figure 4 that the exogenously added proteoglycan also inhibited collagen-gel contraction in cells not expressing recombinant decorin.

Effect of decorin on collagen-gel contraction by normal skin fibroblasts

The studies described above, using CHO cells, indicated that decorin may play an inhibitory role during collagen-gel retraction. However, as non-mesenchymal cells, CHO cells have the disadvantage that serum is required and that rather large cell numbers are needed to achieve gel contraction within a couple of days. Additional experiments were therefore performed using cultured human skin fibroblasts. In contrast with previous reports [34], these cells are able to achieve collagen-gel contraction in the absence of fetal calf serum, albeit at lower velocity (Figure 5). This is in accordance with the observation that collagen-gel contraction by fibroblasts requires cellular but not plasma fibronectin [6].

Since it is difficult to control the penetration of decorin through a contracted collagen gel, the proteoglycan was added together with all the other components at the beginning of the experiment. It can be seen in Figure 6 (left panel) that, in

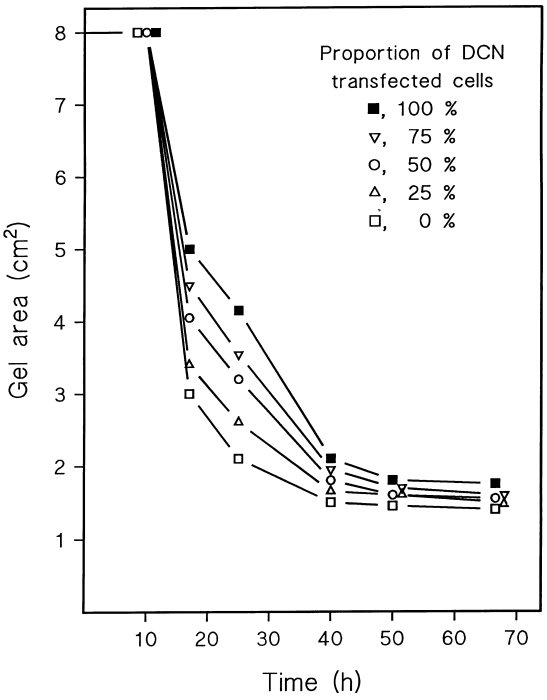

Figure 3 Collagen-gel contraction by mixtures of decorin-transfected and control CHO cells

A total of 450 000 CHO cells were used for incorporation into a single collagen lattice, but the proportion of decorin (DCN)-transfected cells varied as indicated.

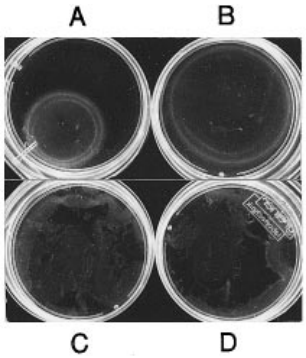

Figure 4 Effect of decorin on collagen-gel contraction by CHO cells

Decorin-expressing cells (**B,D**) and cells not transfected with decorin cDNA (**A,C**) were incubated for 48 h in the absence (**A,B**) or presence (**C,D**) of decorin (50 μg/ml).

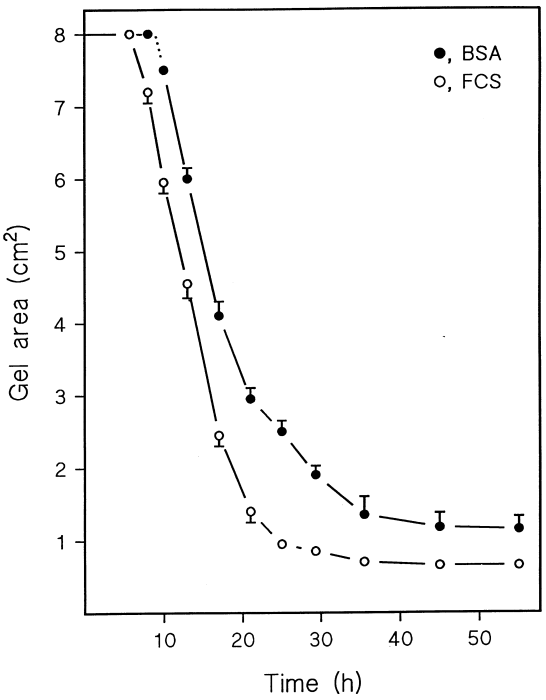

Figure 5 Effect of BSA (●) and fetal calf serum (FCS; ○) on collagen-gel contraction by skin fibroblasts

Fibroblasts (150 000) were mixed with collagen as described in the Experimental section. The final mixture contained either 10% (v/v) fetal calf serum or 1% (w/v) BSA. The range of data of triplicate assays with BSA and of duplicate assays with fetal calf serum is indicated by the bars.

fibroblast-populated collagen lattices also, decorin at doses above 10 μg/dish led to a delayed contraction. The final diameter was somewhat larger too.

The glycosaminoglycan chain of decorin alone was not responsible for the delayed collagen-gel retraction. Even at the highest dose of dermatan sulphate, which was prepared from pig skin decorin by β-elimination, neither the kinetics of gel retraction nor the final diameter were altered compared with the untreated control (Figure 6, right panel).

In a further set of experiments, chondroitin ABC lyase, a chondroitin- and dermatan sulphate-degrading enzyme, was added at the beginning of the experiment. As shown in Figure 7, the enzymic degradation of endogenously formed chondroitin and dermatan sulphate had little influence, if any at all, on the kinetics of collagen-gel contraction. In a parallel experiment, cultures additionally contained [^{35}S]methionine (60 μCi/ml). After 12 h, medium was removed and replaced by fresh [^{35}S]methionine-containing medium to avoid depletion of the labelled precursor pools. After a further 12 h, the gels were dissolved by collagenase treatment, and decorin was recovered by immunoprecipitation. Only core protein and no intact decorin could be visualized after SDS/PAGE and fluorography (results not shown).

As chondroitin ABC lyase had no effect on collagen-lattice contraction, the effect of decorin core protein could be studied directly. Dermatan sulphate chains were enzymically degraded, and the core protein was added together with the enzyme at the beginning of the experiment. As shown in Figure 8, the core protein alone exhibited a gel-contraction-delaying effect, although it was not as effective as the intact proteoglycan. In other experiments inhibition was observed during the initial 24 h only.

Fibroblasts with anomalous decorin metabolism and collagen-gel retraction

We have previously described a patient whose fibroblasts were characterized by a 95% reduction in galactosyltransferase activity *in vitro* and by the secretion of a considerable proportion (about 50%) of glycosaminoglycan-free decorin core protein

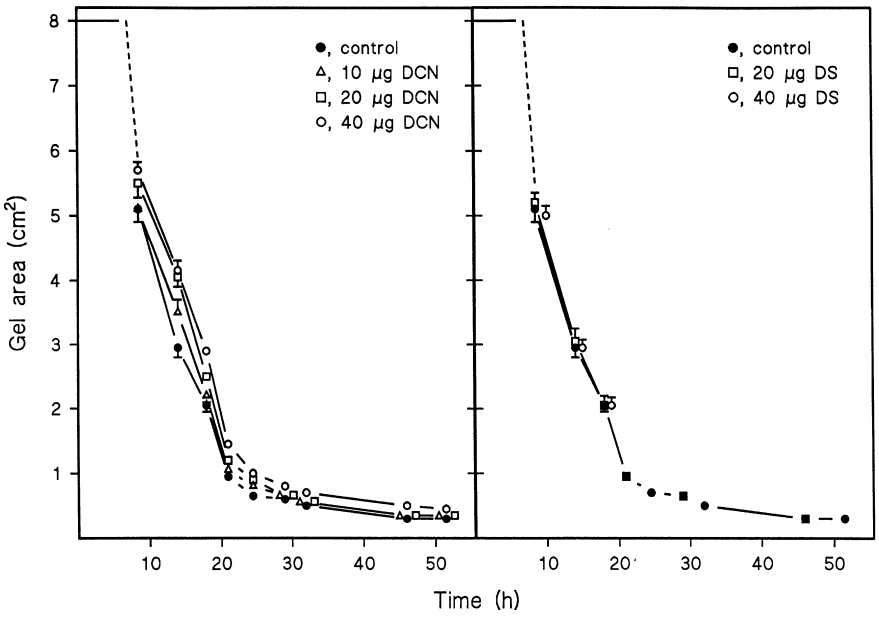

Figure 6 Effect of decorin (left) and protein-free dermatan sulphate chains (right) on collagen-gel contraction by skin fibroblasts

The indicated quantities of decorin (DCN) and dermatan sulphate (DS) were added to cultures maintained under serum-free conditions. The bars indicate the range of data of triplicate assays of control cultures and of duplicate assays of lattices containing decorin and dermatan sulphate.

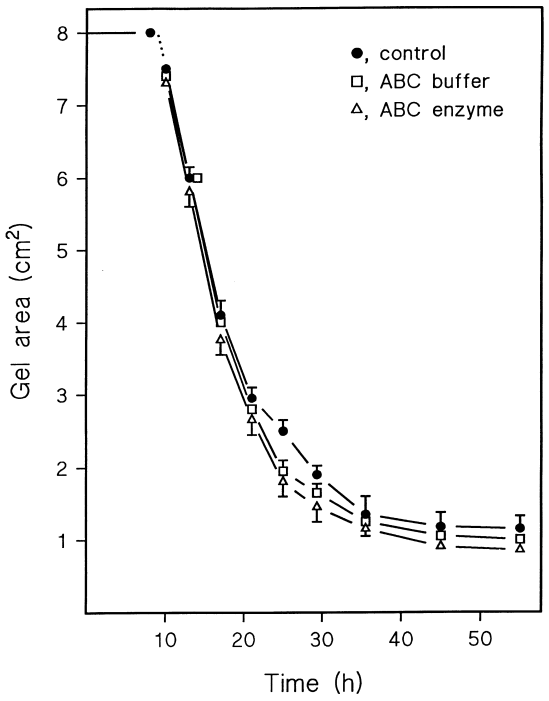

Figure 7 Effect of chondroitin ABC lyase on collagen-gel contraction by skin fibroblasts

A 15 μl volume of 50 mM Tris/HCl, pH 8.0, containing 60 mM sodium acetate, 60 mM NaCl, 0.01% BSA and 150 m-units of the enzyme or enzyme-free buffer was added to the fibroblast suspension at the beginning of the experiment. The bars indicate the range of data of triplicate assays without addition of enzyme or enzyme-free buffer and of duplicate assays with these additions.

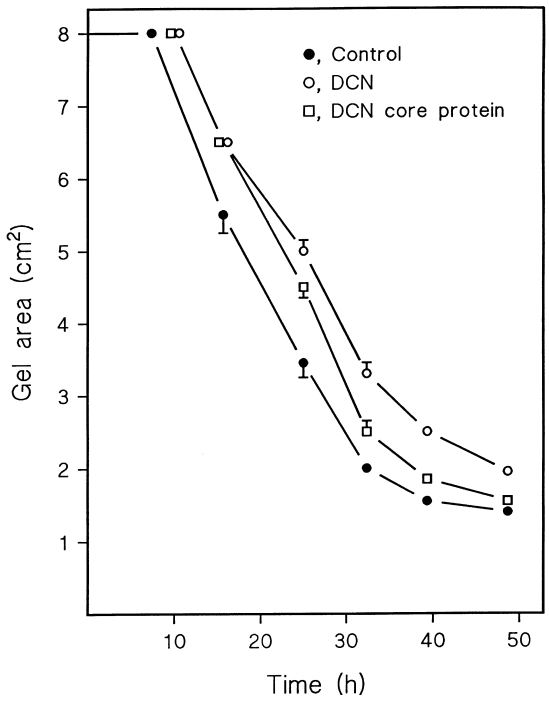

Figure 8 Effect of decorin core protein on collagen-gel contraction by skin fibroblasts

Decorin (DCN; 50 μg) was treated with chondroitin ABC lyase or buffer alone for 2 h at 37 °C as described in the legend of Figure 7 before the digest was added to the fibroblast suspension. The respective amount of enzyme-free buffer without decorin was added to the control. The range of duplicate values is indicated by the bars.

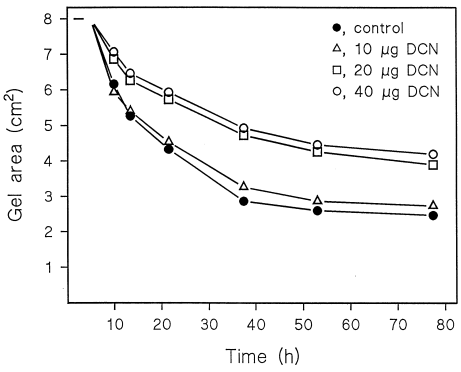

Figure 9 Effect of decorin on collagen-gel contraction by fibroblasts from a patient with progeroidal syndrome and galactosyltransferase I deficiency

The indicated quantities of decorin (DCN) were added to cultures maintained under serum-free conditions. The experiments were performed three times. As the absolute values obtained in these independent experiments varied, no standard deviations can be given. Similar results were, however, obtained in all three experiments.

[27]. No anomaly in the synthesis of versican and heparan sulphate proteins was observed, but small quantities of glycosaminoglycan-free biglycan had been found [35]. Fibroblasts from this patient had a considerably delayed capability for collagen-gel retraction when assayed both at an early (6th) passage (not shown) and a late (24th) passage (Figure 9). Exogenously added decorin delayed this process further, even at small doses.

Self-association of dermatan sulphate

The data presented in the previous sections suggested a modulatory role for intact decorin but not for free glycosaminoglycan chains in the process of collagen-gel contraction. However, a secondary role of dermatan sulphate chains also appeared to be possible because of the behaviour of galactosyltransferase I-deficient cells. Since self-association of dermatan sulphate chains had been described [24,36], gel-filtration experiments were performed under conditions promoting such self-association. On a Sepharose CL-6B column, intact [^{35}S]sulphate-labelled decorin was eluted with a K_{av} value of 0.39, and dermatan sulphate chains derived therefrom by β-elimination were eluted with a K_{av} value of 0.46 (Figure 10a). The labelled protein-free chains were then mixed with 20 μg of unlabelled decorin before chromatography to test for a shift in the elution position. Such a shift would be expected to occur upon association of labelled chains with unlabelled proteoglycan. However, the elution position remained unaltered (Figure 10b). As it was possible that the correct tertiary structure of dermatan sulphate [26,37] was lost under the strongly alkaline conditions of β-elimination, the glycosaminoglycan chain was released by cathepsin C, which successively removes N-terminal dipeptides so that the polysaccharide remains linked with an Ala-Ser residue only. A comparison of the elution profiles shown in Figures 10(c) and 10(d) indicates, however, that the K_{av} value of the glycosaminoglycan chain was 0.46 in both the presence and absence of decorin.

Different results were obtained when trypsin was used to digest the proteoglycan yielding a polysaccharide that is linked with at least 14 amino acids. Figures 11(a) and 11(b) show that the K_{av} value of 0.46 is shifted to 0.42 in the presence of unlabelled

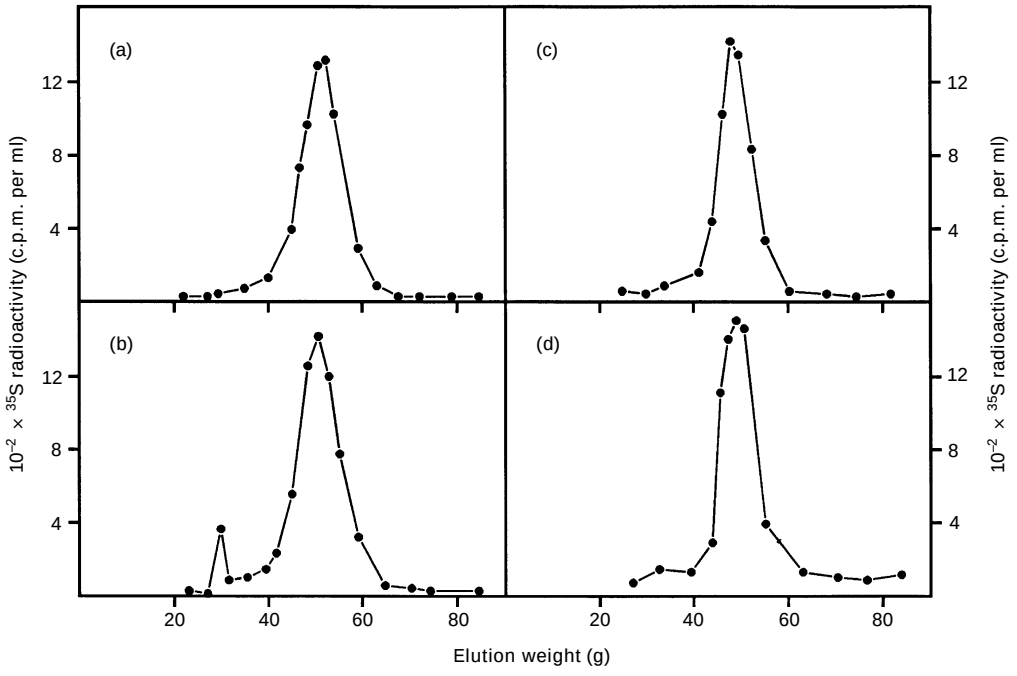

Figure 10 Chromatography on Sepharose CL-6B of [^{35}S]sulphate-labelled glycosaminoglycans in the absence (A,C) or presence (B,D) of 20 μg of unlabelled decorin

The glycosaminoglycan chains were either prepared by β-elimination (**A,B**) or by digestion with cathepsin C (**C,D**). The column was equilibrated and eluted with 0.25 M sodium acetate buffer, pH 7.0, containing 0.05% BSA and 0.1% Triton X-100. As the flow rates were not absolutely constant with time, elution positions were obtained by weighing each fraction: the elution weight for Dextran Blue was 30.1 g and that for [^{35}S]sulphate 71.4 g.

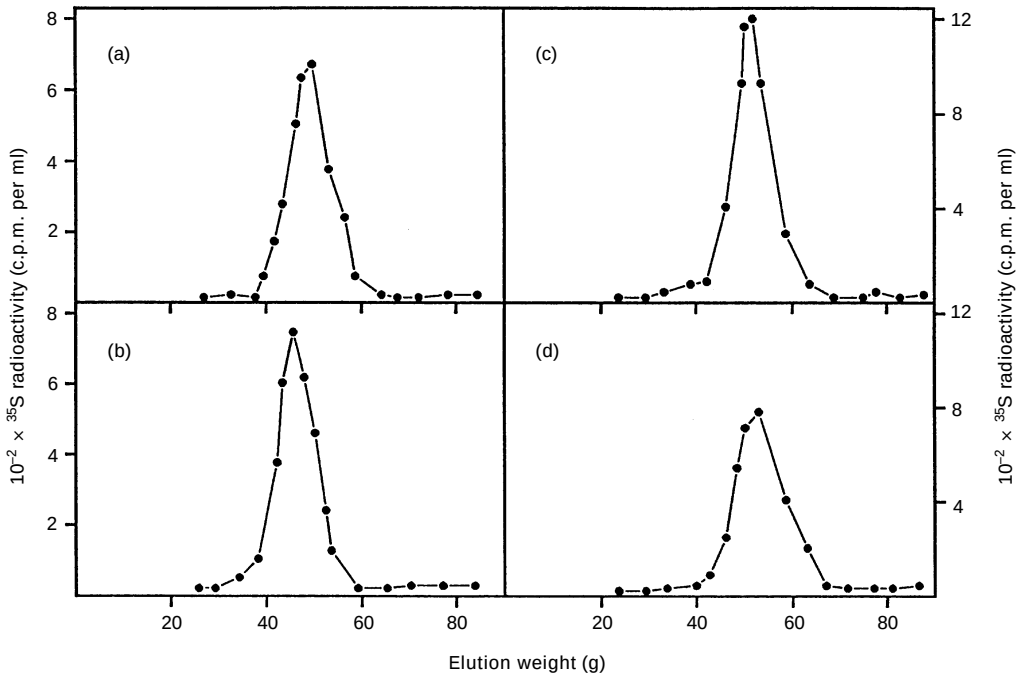

Figure 11 Chromatography on Sepharose CL-6B of [^{35}S]sulphate-labelled glycosaminoglycans obtained by trypsin digestion in the absence (A,C) or presence (B,D) of 20 μg of unlabelled decorin

In (**A**) and (**B**) the elution conditions were as described in the legend to Figure 10. In (**C**) and (**D**) 4 M guanidinium chloride was included in the elution buffer, and the elution weights for Dextran Blue and [^{35}S]sulphate were 35.2 g and 81.7 g respectively.

decorin. Chromatography of labelled trypsin-released chains on Sepharose CL-6B in the presence of 4 M guanidinium chloride yielded a K_{av} of 0.38 regardless of the presence or absence of unlabelled decorin (Figures 11c and 11d). Labelled decorin exhibited a K_{av} value of 0.26 under these conditions.

DISCUSSION

The main finding of the present investigation is that, in cell-populated collagen lattices, decorin has an inhibitory effect on the contraction of the gel. An abstract published recently mentions a similar finding [38]. Collagen-gel contraction is considered to be an *in vitro* model of wound contraction. In a study on the healing of human mucosal wounds it was shown that, in the granulation tissue, the first signs of decorin expression could be seen in the same area as the first collagen bundles. Both macromolecules were expressed when the epithelial sheets from the wound margin had just closed the wound gap [39]. Subsequent tissue condensation could therefore be dependent, in part, on the quantity of decorin, a greater expression of decorin leading to a softer extracellular matrix.

Considering the mechanism by which decorin influences collagen-gel contraction, the following findings should be put together. Protein-free dermatan sulphate chains were ineffective when added in quantities similar to those of decorin. This lack of effect has also been found in previous investigations [2], and additional support comes from the absence of effects of chondroitin ABC lyase digestion. Furthermore dermatan sulphate exhibited only a minor effect on the physicochemical properties of reconstituted collagen [40]. Dermatan sulphate-free core protein caused a delay during early stages of the contraction process. The absence of longer-lasting effects, however, could be explained by the assumption of rapid degradation of the glycosaminoglycan-free core protein. Such an instability had been inferred indirectly from the low decorin core protein content in the skin of the patient with galactosyltransferase I deficiency [35]. It is therefore suggested that the effect of decorin on collagen-gel contraction is mediated primarily by its core protein, although an influence of the core-bound dermatan sulphate chain cannot be completely excluded. There are at least two, not mutually exclusive, explanations of the effects. Decorin is not known to bind to an integrin receptor. However, it has been shown to interact with the cell-binding domain and with both heparin-binding domains of fibronectin and to interfere with cell adhesion to a fibronectin substrate [32,41]. Since collagen-gel contraction requires the expression of cellular fibronectin [6], fibronectin-dependent cellular activities required for gel contraction to occur may be inhibited by decorin. Another explanation is based on the assumption that the mobility of hydrated collagen fibrils within the gel may be retarded when they are saturated with decorin. The opposite phenomenon was observed in similar experiments to those described here on addition of type-XII and -XIV collagens. These collagens localize near the surface of banded collagen fibrils and increase their mobility [42]. Type-XIV collagen at least has been shown to interact with the dermatan sulphate chain of decorin [43].

On the basis of previous findings [24,25,36] it was originally hypothesized that a potential influence of decorin on collagen-gel contraction is mediated by the self-association of dermatan sulphate chains which occurs optimally when alternating glucuronic acid- and iduronic acid-containing oligosaccharide units are present along the polysaccharide chains [36]. In experiments using decorin from skin fibroblasts as a probe for self-association and making use of the simple gel-filtration assay described by

Fransson [24], no interactions depending exclusively on interglycosaminoglycan forces were observed. Instead, a shift in the elution behaviour attributable to self-association was observed only when dermatan sulphate chains prepared by tryptic digestion were used. Since such chains contain at least 14 amino acids, five of them being lysine or arginine, ionic interactions between intact decorin and the dermatan sulphate peptides cannot be ruled out. An interaction between the peptide and the intact core protein represents a further possible explanation. It is also noteworthy that, in the previous studies on self-association of dermatan sulphate, proteolytically prepared glycosaminoglycans had always been used and therefore a contribution of the peptide moiety to the interactions cannot be excluded.

Decorin has recently attracted great attention because of its TGF-β-neutralizing activity and its potential use as a therapeutic agent in fibrotic diseases [44,45]. However, decorin inhibits only selected and not all effects of TGF-β [46]. The inhibitory effect of decorin on the contraction of fibroblast-populated collagen lattices suggests a beneficial effect of decorin on fibrotic processes which is independent of the effect of the proteoglycan on TGF-β. The potential use, however, needs to be verified in an *in vivo* model of fibrosis.

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