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# Role of decorin on *in vitro* fibrillogenesis of type I collagen

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**Tendon and corneal decorins are differently iduronated dermatan sulphate/proteoglycan (DS/PG) and the biochemical parameter that differentiates type I collagens is the hydroxylysine glycoside content. We have examined the effect of tendon and corneal decorins on the individual phases (tlag, dA/dt) of differently glycosylated type I collagens fibril formation, at molar ratios PG:collagen monomer ranging from 0.15 : 1 to 0.45 : 1. The results obtained indicate that decorins exert a different effect on the individual phases of fibril formation, correlated to the degree of glycosylation of collagen: at the same PG:collagen ratio the fibril formation of highly glycosylated corneal collagen is more efficiently inhibited than that of the poorly glycosylated one (tendon). Moreover tendon and corneal decorins exert a higher control on the fibrillogenesis of homologous collagen with respect to the heterologous one. These data suggest a possible tissue-specificity of the interaction decorin/type I collagen correlated to the structure of the PG and collagen present in extracellular matrices.**

***Keywords*: proteoglycans, extracellular matrix, collagen, decorin, collagen fibrillogenesis**

## Introduction

Connective tissues contain collagen and proteoglycans (PGs) as predominant components; each of these is now recognized to represent large families of distinct molecules. Decorin, a low molecular weight PG that contains a chondroitin/dermatan sulphate chain and has a leucine rich 45 kDa core protein, is present in the extracellular matrix of numerous interstitial tissues and it is characterized by different degrees of iduronation of the saccharidic moiety [1]. Decorin binds several connective tissues macromolecules and modulates their functions. In particular it binds type I collagen inhibiting its *in vitro* fibrillogenesis. Type I collagen is a heterotrimer composed of two identical alpha 1(I) and one alpha 2(I) chains providing the major mechanical strength in different connective tissues. It was previously proved that *in vitro* fibril formation from collagen of various tissues takes place with different kinetics and results in the formation of fibrils of various diameters; the final size of fibril is correlated to the degree of hydroxylysine glycosylation of type I collagen [2]. The assumed location of decorin on collagen fibrils *in vivo* [3] and its ability to retard collagen fibrillogenesis *in vitro* [4] suggest that it has a role in the collagen network organization and in the maintenance of tissue integrity. The present study was undertaken to characterize the role of tendon and corneal decorins in the control of fibril formation of type I collagens from tissues with different ultrastructural architecture (tendon and cornea). Tendon and corneal decorins are differently iduronated [5] and the only biochemical parameter that differentiates type I collagens in these tissues is their hydroxylysine glycoside content. We have examined the effect of decorins on the individual phases of type I collagens fibril formation (tlag, dA/dt) at molar ratios PG:collagen monomer ranging from 0.15:1 to 0.45:1.

## Material and methods

### Collagen purification

Acid-soluble type I collagen was prepared from tissues (adult bovine tendon and cornea) by extraction with 0.5 M acetic acid [6, 7]. To avoid proteolysis, acid extraction was done in the presence of proteinase inhibitors [8]. The preparations were stored freeze-dried and the collagen concentration was quantified by hydroxyproline determination in samples after hydrolysis in 6 M HCl for 24 h at 110 °C [9]. By this assay, control samples contained 9–11 μg of hydroxyproline per 100 μg of collagen. Slab SDS polyacrylamide gel electrophoresis was performed with a 3% stacking gel

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and a 6% separating gel as described by Laemmli [10]. Hydroxylysine glycosides were determined in purified collagens according to Isamura *et al.* [11] using a LKB 2051 AminoacidAnalyzer. The structural morphology of purified type I collagens, after *in vitro* fibrillogenesis was checked by scanning electron microscopy (SEM). Collagen fibrillogenesis (25 μg) was performed in a Microcon 50 (Amicon) as reported in the section 'Collagen Fibrillogenesis' in the presence of a golden grid (300 mesh). Fibrils so obtained were fixed with 2% glutaraldehyde for 30 min, centrifuged and washed with water. The grid supporting collagen fibril was covered with gold, chrome or coal-platinum by rotary shadowing technique (Balzer BAF 301, two EK 552 E.B.G.) at − 85 °C for 50 s. The thickness of covering layers were 20, 10 and 2 nm respectively.

## Decorin purification

Decorin was isolated from a 4 M guanidine hydrochloride extract of adult bovine tendon (tensional region of bovine deep flexor tendon) and cornea, and purified as described elsewhere [4]. The PG was stored in 4 M guanidine hydrochloride 0.05 M sodium acetate, pH 5.8 and frozen as aliquots. Before using decorin solutions were dialysed against distilled water and then diluted with the appropriate buffer. Decorin core proteins were obtained by Chondroitinase ABC treatment [12] and characterized by SDS-PAGE.

## Fibrillogenesis assay

Collagen was redissolved in 5 mM acetic acid at 1 mg ml$^{-1}$ and stored at 4 °C until utilized. For a final volume of 0.5 ml, 0.25 ml of 20 mM sodium phosphate, 280 mM NaCl, 5 mM KCl, pH 7.4 was combined with 0.125 ml of proteoglycan and 0.125 ml of collagen. Fibrillogenesis was initiated by rapidly warming the solution to 33 °C in a thermostated cell. The kinetics of fibril formation was followed turbidimetrically at 313 nm; the turbidity curve is characterized by the following parameters: ΔA, the total absorbance change in the plateau; dA/dt, the rate of fibril formation measured from the slope of the linear segment of sigmoidal curve; tlag, the lag time between the start of incubation and the absorbance increase (Figure 1).

## Results and discussion

Acid soluble type I collagens were electrophoresed by SDS-PAGE giving the typical patterns in which alpha 1(I) and alpha 2(I) chains can be clearly distinguished as well as beta dimers and gamma trimers (Figure 2). The amount of covalently cross-linked collagen oligomers was sufficiently low (about 10%) not to affect the kinetics of collagen fibrillogenesis [13]. The hydroxylysine glycoside content (Table 1) is significantly higher in corneal collagen than in tendon one. Figure 3 shows the appearance of tendon and corneal

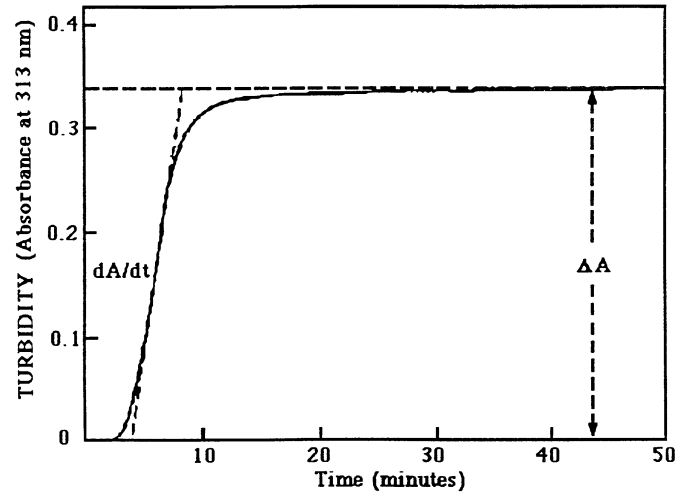


**Figure 1.** Kinetics of type I collagen fibril assembly as demonstrated by turbidimetry.

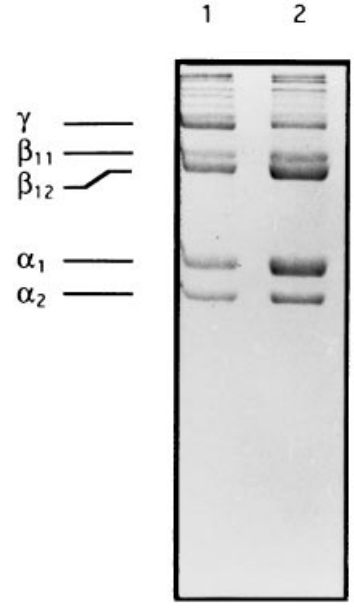


**Figure 2.** SDS-polyacrylamide gel electrophoresis of type I collagen. 10 micrograms of denatured type I collagens were separated per lane on 6% SDS-PAGE gels and stained with Coomassie Blue. Lane 1: tendon collagen; lane 2: corneal collagen. Alpha 'monomers' and beta 'dimers' are clearly resolved from each other and from gamma 'trimers'.

**Table 1.** Hydroxylysine glycosylation of tendon and corneal type I collagens. *Collagen HyLys content is expressed as nmoles/mg of collagen

| *Source* | *Total HyLys** | *%Glycosylation* |
|---|---|---|
| Tendon | 59.5 | 11.3 |
| Cornea | 49.8 | 43.0 |

decorins on SDS-PAGE before and after Chase ABC treatment; biglycan contamination can be excluded since it has been reported that it is absent in cornea [14] and in adult tensional tendons [15–17].

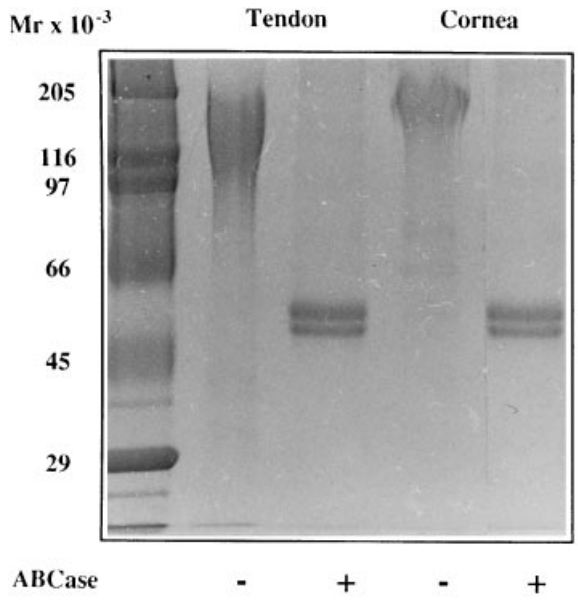


**Figure 3.** SDS-Page of tendon and corneal decorins.

In our experimental conditions *in vitro* fibrillogenesis results in formation of typical collagen fibrils, as proved by evidence from scanning electron microscopy (Fig. 4); however, the growth curves of tendon and corneal collagen are characterized by different lag times and slopes. The faster fibril formation occurs with poorly glycosylated tendon collagen, the slower growth with highly glycosylated corneal collagen. Type I collagen fibril self-association was also performed in the presence of decorins at different PG:collagen monomer molar ratio. The results indicate that decorins inhibit the process of *in vitro* fibril formation from the acid soluble collagens. Tendon and corneal decorins show a different effect on the individual phases of fibril formation, correlated to the degree of glycosylation of collagens (Table 2). At the same PG:collagen ratio fibril formation of highly glycosylated corneal collagen is more strongly affected than that of poorly glycosylated tendon collagen. Moreover tendon and corneal decorins exert a higher control on the fibrillogenesis of homologous collagen with respect to the heterologous one. These data suggest a possible tissue-specificity of the interaction decorin/type I collagen correlated to the structural characteristics of the PG (iduronation degree, glucidic chains length and N-linked oligosaccharides) and collagen (glycosylation degree) present in the extracellular matrix. The whole PG may contribute to the final tissue organization; core protein is able to bind

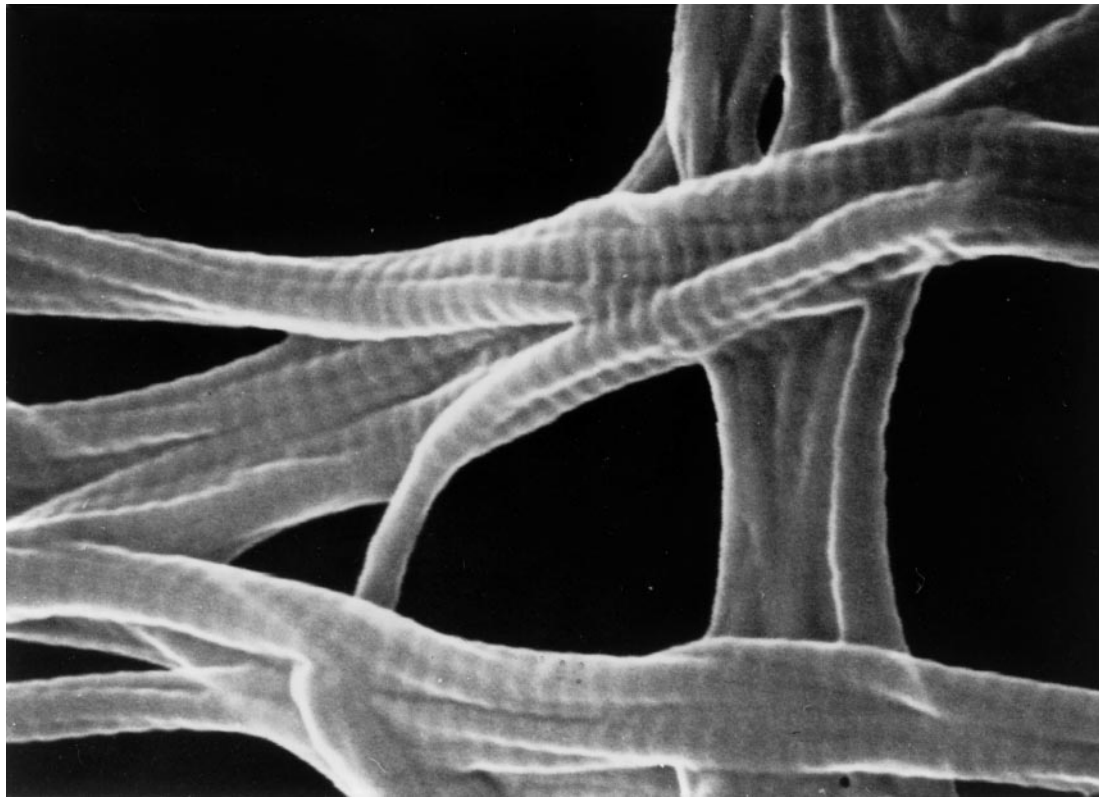

**Figure 4.** Scanning electron microscopy of collagen fibrils obtained by *in vitro* fibrillogenesis (30 000 × 2.6).

**Table 2.** Percentage modification of kinetic parameters of *in vitro* fibrillogenesis of tendon and corneal type I collagens in the presence of homologous and heterologous decorin. (A) Tendon collagen was incubated in the presence of tendon and corneal decorin at the reported molar ratios. The % increase of the lag time and the % decrease of the slope of the curve are reported. (B) Corneal collagen fibrillogenesis was investigated in the same experimental conditions.

| *Molar ratio decorin:collagen* | *% increase tlag* | | *% Decrease dAldt* | |
|---|---|---|---|---|
| | Tendon | Cornea | Tendon | Cornea |
| A) Tendon collagen | | | | |
| 0.15 | 26.4 ± 7.3 | 0.0 | 26.8 ± 3.0 | 0.8 ± 1.0 |
| 0.30 | 59.9 ± 0.9 | 0.0 | 28.9 ± 1.3 | 18.3 ± 3.0 |
| 0.45 | 57.4 ± 3.1 | 0.0 | 29.4 ± 2.8 | 20.9 ± 8.1 |
| B) Corneal collagen | | | | |
| 0.15 | 110.0 ± 10.4 | 52.8 ± 15.6 | 34.2 ± 2.1 | 79.6 ± 8.3 |
| 0.30 | 194.0 ± 12.0 | 77.3 ± 8.9 | 66.5 ± 2.1 | 87.8 ± 1.3 |
| 0.45 | 254.0 ± 9.5 | 314.0 ± 46.6 | 79.2 ± 1.5 | 96.3 ± 1.0 |

collagen fibres, but the distance between adjacent fibrils may be controlled by the glycosaminoglycans chains of collagen bound to decorin. In cornea stroma, in particular, collagen interfibrillar distance is possibly due to self association of the glycosaminoglycans chains of decorin molecules bound on opposite collagen fibrils. In this tissue decorin is thus a bifunctional cross-linking molecule, with the core protein bound to collagen and glycosaminoglycans chains of opposite molecules self-aggregating [18]. These results confirm that decorin plays a pivotal role in the assembly of collagen fibrils, its effect being strongly dependent on the iduronation degree of the DS chain and on the glycoside content of collagen molecule.

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## References

1 Heinegård D, Olberg A (1993) In *Connective Tissue and its Heritable Disorders* (Royce PM ed.), pp 189–209. New York: Wiley Liss Inc.
2 Valli M, Leonardi L, Strocchi R, Guizzardi S, Ruggeri A, Balduini C (1986) *Conn Tiss Res* **15**: 235–44.
3 Scott JE (1993) In *Dermatan Sulphate Proteoglycans*: *Chemistry*, *Biology*, *Chemical Pathology* (Scott JE, ed.), pp 165–82, Portland Press Ltd.
4 Vogel KG, Trotter JA (1987) *Collagen Rel Res* **7**: 105–14.
5 Rosenberg L, Tang LH, Choi HU, Johnson TL (1993) In *Dermatan Sulphate Proteoglycans*: *Chemistry*, *Biology*, *Chemical Pathology* (Scott JE ed.), pp 225–39. Portland Press Ltd.
6 Vogel GK, Paulsson M, Heinegård D (1984) *Biochem J* **223**: 587–97.
7 Chandrakasan G, Torchia DA, Piez KA (1976) *J Biol Chem* **251**: 6061–67.
8 Hedbom E, Heinegård D (1993) *J Biol Chem* **268**: 27307–12.
9 Huszar H, Maiocco J, Naftolin E (1985) *Anal Biochem* **105**: 424–28
10 Laemmli UK (1970) *Nature* **227**: 680–85.
11 Isemura M, Takahashy K, Ikenaka T (1976) *J Biochem* (*Japan*) **80**: 653–58.
12 Denti A, Sini P, Tira ME, Balduini C (1995) *Thromb Res* **79**: 187–98.
13 Na GC, Butz LJ, Bailey DG, Carroll RJ (1986) *Biochemistry* **25**: 958–66.
14 Blochberger TC, Cornuet PK, Hassel JR (1992) *J Biol Chem* **267**: 20613–19.
15 Vogel KG, Evanko SP (1987) *J Biol Chem* **262**: 13607–13.
16 Evanko SP, Vogel KG (1990) *Matrix* **10**: 420–36.
17 Svensson L, Heinegård D, Oldberg Å (1995) *J Biol Chem* **270**: 20712–20.
18 Scott JE (1995) *J Anat* **187**: 259–69.

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