Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 36–42
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Decorin from different bovine tissues: Study of glycosaminoglycan chain by PAGEFS

Manuela Viola [a], Evgenia G. Karousou [a], Davide Vigetti [a], Anna Genasetti [a], Francesco Pallotti [a], Gianni F. Guidetti [b], Enrica Tira [b], Giancarlo De Luca [a], Alberto Passi [a,*]

[a] *Department of Experimental and Clinical Biomedical Sciences, University of Insubria, Varese, Italy*
[b] *Department of Biochemistry "A.A. Castellani", University of Pavia, Pavia, Italy*

Received 4 July 2005; received in revised form 4 October 2005; accepted 6 October 2005
Available online 15 November 2005

Abstract

The sulphation pattern of glycosaminoglycan (GAG) plays a critical role in biological functions of proteoglycans. In this study, we showed that decorins from different bovine tissues present specific sulphation pattern coupled with peculiar biological activity. In order to elucidate chemical structure of decorin glycosaminoglycan chains, we improved an electrophoretic method to analyse fluorescent disaccharides from dermatan/chondroitin sulphate GAG chains. The disaccharide separation is based on minigels, and this technique was able to define the polysaccharide chain composition in terms of sulphated and not sulphated disaccharides. This approach allowed not only the measurement of few picomoles of material, but it also permits a rapid qualitative analysis of the GAG chains. Data obtained by PAGEFS indicate that the sulphation pattern of GAG is tissue specific and this finding may explain the different binding properties to von Willebrand factor of decorins.

Keywords: Proteoglycan; Glycosaminoglycan; Carbohydrate electrophoresis; Decorin

1. Introduction

Proteoglycans are macromolecules widely distributed in nature, showing great variability in structure and functions; they play important roles in extracellular matrix (ECM) as well as on cell membranes both for maintenance of the tissue structure and cell metabolism. These peculiarities of proteoglycans depend on their structural complexity, hence these molecules have a core protein and at least one sulphated polysaccharide chain, the glycosaminoglycan (GAG) chain. GAG chains are covalently linked to the core protein and possess a great variety of chemical species and structures. GAGs are classified as galactosaminoglicuronans (dermatansulphate, and chondroitinsulphate) and glucosaminoglicuronans (heparansulphate and heparin), characterized by the presence of disaccharide units, these latter composed by a glucuronic acid covalently linked with a glycosidic bond to a mono- or di-sulphated hexosamine. The only GAG without sulphated hexosamine and without a core protein is hyaluronan.

One of the most studied proteoglycan in literature is decorin, a member of the family of small leucine-rich proteoglycans

Abbreviations: ABCase, chondroitinase ABC; AMAC, 2-aminoacridone; GAGs, glycosaminoglycans; PAGEFS, polyacrylamide gel electrophoresis of fluorophore labelled saccharides; HPLC, high-performance liquid chromatography; UA, uronic acid; Δdi-nonS_{CS}, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-4-D-galactose; Δdi-nonS_{HA}, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-4-D-glucose; Δdi-mono4*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-4-*O*-sulpho-D-galactose; Δdi-mono6*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-6-*O*-sulpho-D-galactose; Δdi-mono2*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-2-*O*-sulpho-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose; Δdi-di(2,4)*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-2-*O*-sulpho-α-L-*threo*-hex-4-enopyranosyluronic acid)-4-*O*-sulpho-D-galactose; Δdi-di(2,6)*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-2-*O*-sulpho-α-L-*threo*-hex-4-enopyranosyluronic acid)-6-*O*-sulpho-D-galactose; Δdi-di(4,6)*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-4,6-*O*-sulpho-D-galactose; Δdi-tri(2,4,6)*S*, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-2-*O*-sulpho-α-L-*threo*-hex-4-enopyranosyluronic acid)-4,6-*O*-sulpho-D-galactose

* Corresponding author. Fax: +39 0332 217119.
E-mail address: alberto.passi@uninsubria.it (A. Passi).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.10.010

(SLRP) [1–3]. Decorin plays a key role in regulating collagen fibril formation and the spatial arrangement of collagen fibres in the matrix [4]; moreover, decorin has also the ability to bind cytokines as transforming growth factor-β [5] and molecules of the epidermal growth factor receptor family [6,7].

The role of GAG chain in the turnover of the entire proteoglycan molecule was recently underlined [8] as well as its role in biology of different class of cancer [9]. Furthermore, decorin has been proposed as tumor suppressor in cancer cells during malignant transformation, and overexpression of decorin in the tumor ECM, probably by myofibroblasts, has been postulated to represent a response of the host to invading cancer cells in an attempt to avert tumor spread [10–13].

The core protein domain of various proteoglycans was deeply studied and characterized in terms of gene sequence, splicing variants [14] and crystallographic properties [1,15]. This approach was sustained by the efficiency of the new techniques of molecular biology and by the increasing importance of core protein in most important biological activities of the cells, including interactions with matrix proteins and growth factors. Nevertheless, the chemical nature and structural aspects of GAGs are not often deeply studied, even though recently it was shown that GAG chains can play a critical role in proteoglycan biological functions [16,26].

In this study we present a new approach developed from the already published PAGEFS [17,25,28], a useful method to obtain a fast and sensitive characterization of the GAG structure. These results were also confirmed using as alternative method the HPLC. PAGEFS applied to decorins suggests that this approach can be an important tool for studying the GAG chains from all proteoglycan containing galactosaminoglicuronan. Moreover, applying this modified PAGEFS method to decorins extracted from various bovine tissues, we were able to improve our information about the GAG chain sulphation pattern and its role in the binding of decorin to von Willebrand factor [16].

2. Materials and methods

2.1. Materials

Standard preparations of Δdi-nonS_{CS}, Δdi-mono6*S*, Δdi-mono4*S*, Δdi-di(2,6)*S*, Δdi-di(4,6)*S*, Δdi-tri(2,4,6)*S*, were all purchased from Seikagaku Corp. (Tokyo, Japan); Δdi-mono2*S* and Δdi-di(2,4)*S* were purchased from Sigma (St. Louis, MO, USA). Protease K (EC 3.4.21.64) was from Finnzymes (Espoo, Finland) and Chondroitinase ABC (EC 4.2.2.4) from Seikagaku Kogyo (Tokyo, Japan). AMAC was obtained from Molecular Probes (Oregon, USA) and $NaBH_3CN$ from Sigma–Aldrich (Steinheim, Germany). Acetonitrile HPLC grade was from Merck (Darmstadt, Germany). Acrylamide, *N*,*N*′-methylenebisacrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium persulphate were obtained from Bio-Rad (Richmond, CA). Peroxidase-conjugated anti-VWF antibody was from Dako (Lostrup, Denmark). All aqueous solutions were prepared using water filtered through a Milli-Q water system (Millipore). All other chemicals used were of analytical reagent grade.

2.2. Methods

2.2.1. Decorins purification

Decorins were extracted from three bovine tissues: tendon, aorta and cornea as described elsewhere [21]; the only modification was that after ultracentrifugation in high density cesium chloride three parts at different density were recovered (D1 with density 1.45, D2 1.5 and D3 1.55 g/ml) and in two of them, D1 and D2, was found the proteoglycan decorin, subsequently purified as reported above. Decorins found in D2 fractions from tendon, aorta and cornea were indicated as D2T, D2A and D2C, respectively, whereas in D1 fractions decorins only from aorta and tendon called D1A and D1T, respectively, were found.

Proteoglycans were desalted with the Vivaspin concentrator. Protein content was determined with the Bradford's method [22] and uronic acid (UA) content of the GAG chain with the Bitter and Muir method [23]; purity of decorins samples was tested by electrophoretic analysis in denaturing condition before and after chondroitinase ABC digestion (see below) according to Laemmli [24] followed by gel staining with Coomassie Brilliant Blue R250.

For PAGEFS and HPLC analysis, 10 μg of UA (=50.5 nmol of total disaccharides) from decorin were treated with chondroitinase ABC in 0.1 M ammonium acetate, pH 7.00 1 mU enzyme/μg UA [21] at 37 °C for 16–18 h, centrifuged and the supernatant lyophilized and derivatized.

2.2.2. Derivatization procedure

Derivatization of CS/DS standard and decorin Δ-disaccharides was done as described by Calabro et al. [25], using 2 nmol of each standard Δ-disaccharide or the Δ-disaccharide derived from decorin digestions (10 μg of UA). A 40 μl volume of 12.5 mM AMAC solution in glacial acetic acid/DMSO (3:17, v/v) was added, and samples were incubated for 10–15 min at room temperature. A 40 μl volume of a freshly prepared solution of 1.25 M $NaBH_3CN$ in water was added to each sample followed by an overnight incubation at 37 °C. An appropriate dilution of these samples in ammonium acetate 0.1 M pH 7.0 or the addition of 20% glycerol was used for the HPLC analysis or the PAGEFS, respectively.

2.2.3. HPLC analysis

Separation and analysis of AMAC-derivatives of Δ-disaccharides were done as described by Karousou et al. [17] with a Jasco-Borwin chromatograph system with a fluorophore detector (Jasco FP-920, $\lambda_{ex} = 442$ nm and $\lambda_{em} = 520$ nm). Chromatography was carried out using a reversed phase column (C-18, 4.6 mm × 150 mm, Bischoff) at room temperature, equilibrated with 0.1 M ammonium acetate buffer, pH 7.0, filtered through a 0.22 μm membrane filter. A gradient elution was done using a binary solvent system composed of 0.1 M ammonium acetate buffer, pH 7.0 (eluent A), and acetonitrile (eluent B). The flow rate was 1 ml/min, and the following program was used: pre-run of column with 100% eluent A for 20 min, isocratic

elution with 100% eluent A for 5 min, gradient elution to 30% eluent B for 30 min and from 30 to 50% for 5 min. Sample peaks were identified and quantified comparing the fluorescence spectra with standard Δ-disaccharides, using Jasco-Borwin software.

A 10 μl volume of the derivatized samples (1/10 of the total) was analysed and compared.

2.2.4. PAGEFS

A MiniProtean II or III cell vertical slab gel electrophoresis apparatus (Bio-Rad) was used with 7.2-cm plates, 0.75-mm spacer, and wells of 0.5 cm. The stock solutions were 1.5 M Tris–HCl, pH 8.8. Acrylamide solution *T* 50%/*C* 10% and *T* 50%/*C* 15%. [%*T* refers to the total concentration (w/v) of acrylamide monomer (i.e. acrylamide plus methylenebisacrylamide); %*C* refers to the concentration (w/w) of cross-linker relative to the total monomer.]

A 10 ml volume (for two gels) of *T* 33%/*C* 6.6%, 375 mM Tris–HCl resolving gel buffer solution (final concentrations), was prepared and degassed. Ten microliters of TEMED and a 100 μl of 10% (w/v) ammonium persulphate were added. The solution was carefully mixed before and placed between the glass plates; the gels had a length of at least 6 cm. The stacking gel was prepared in a volume of 5 ml (for two gels) of *T* 20%/*C* 6%, 375 mM Tris–HCl gel buffer solution (final concentrations) with added 5 μl of TEMED and 50 μl of 10% (w/v) ammonium persulphate for the polymerization.

The running buffer was 25 mM Tris–HCl and 192 mM glycine pH 8.3.

A 5 μl volume of each sample (1/20 of the total) was loaded. A marker sample containing bromophenol blue was also run in a well with no sample. Electrophoresis was performed at 4 °C at 180 V for 1 h and then at 400 V since the dye marker was 1.5 cm from the bottom of the gel (∼45 min).

Gels were scanned in a UV-light box using a CCD camera (Gel Doc 2000 System) from Bio-Rad Laboratories (Hercules, CA).

2.2.5. Solid phase binding assay

Solid phase binding assay was performed as described by Guidetti et al. [16]. Briefly, wells of microtiter plates were coated in triplicate with 4 μg of BSA and 4 μg of decorin as protein (in 50 μl of PBS), for 16 h at 4 °C. Upon coating, washings and blocking procedures, plates were incubated with 2 μg of purified VWF in 50 μl of PBS for 2 h at room temperature. Bound VWF was detected by peroxidase-conjugated anti-VWF antibody (1:5000 dilution in PBS containing 1% BSA and 0.05% Tween 20). Absorbance was measured at 490 nm using an ELISA microplate reader.

3. Results

In the present study we analysed the composition of GAG chain from different decorins using a developed electrophoretic method in comparison with traditional HPLC method. The analysis was performed to investigate the role of the sulphation pattern in the interaction of decorin with von Willebrand factor.

Basically, this approach includes GAG specific digestions with chondroitinase ABC to produce disaccharides and, after 2-AMAC labelling, the complete separation of these disaccharides by polyacrylamide gel electrophoresis or HPLC. The PAGEFS separation allowed the analysis by fluorescence detection of the resolved bands at an extraordinary sensitive level in a linear range from 10 to 75 pmol for each disaccharides (data not shown), according to the results described elsewhere [17].

As shown in Fig. 1, we were able to separate seven disaccharides obtained from chondroitin/dermatan GAGs using high percentage polyacrylamide separating gel and Tris–HCl buffers (Fig. 1, lane 7) but also the unusual Δdi-mono2*S* disaccharide was resolved from the other Δmono-disaccharides by this technique (Fig. 1, lane 8). Compared to the other methods already described in literature for no sulphated GAG [17], the peculiarity of this electrophoretic procedure is the salt formulation of the buffers. In fact, Tris–HCl buffers used in gel and as running buffer better separated the disaccharides than Tris–borate buffers described for no sulphated and low sulphated disaccharide electrophoresis separation [17,18]. A Tris–HCl buffer was used by Seyfried et al. to resolve a mixture of oligosaccharides from hyaluronan, even if in that case the separation was mainly on the basis of the molecular dimension and not by the charge of the samples [27]; in our condition we also separated structural isomers: Δmono- and Δdi-sulphate disaccharides differing in the position of sulphation (e.g. Δdi-di(4,6)*S* and Δdi-di(2,6)*S* or Δdi-mono4*S* and Δdi-mono6*S*), whereas the separation of non-sulphated isomers requires Tris–borate PAGEFS.

In our system all disaccharide bands were clearly separated as shown in Fig. 1, both in single and in mixture preparation. As expected, the bands with higher sulphate content showed a faster migration, whereas the bands with the same sulphate content showed slightly different migration. Therefore, in this case the separation is probably due to the different spatial geometry of the sulphate groups. The bands represented 2-AMAC labelled disaccharide standards that can be obtained from chondroitinsulphate and dermatansulphate GAG chains. The extreme sensitivity of the system allowed to detect some impurities in commercial standards, as shown, for example, in Fig. 1 (lane 4), where Δdi-di(4,6)*S* showed contamination of traces of Δdi-mono4*S*.

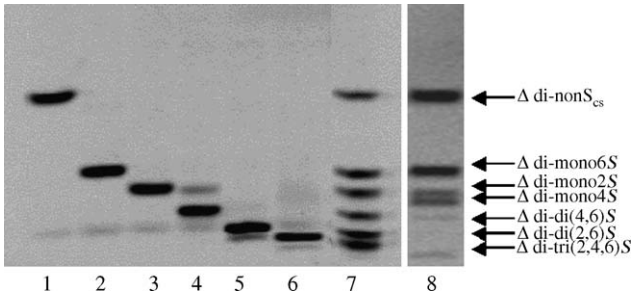


Fig. 1. PAGEFS of standard CS disaccharides; 40 pmol of each 2-AMAC-derivatized disaccharide was loaded in the gel: lane 1, Δdi-nonS_{CS}; lane 2, Δdi-mono6*S*; lane 3, Δdi-mono4*S*; lane 4, Δdi-di(4,6)*S*; lane 5, Δdi-di(2,6)*S*; lane 6, Δdi-tri(2,4,6)*S*; lane 7, mix of all the CS disaccharides; lane 8, mix of Δdi-mono6*S*, Δdi-mono4*S*, Δdi-mono2*S*.

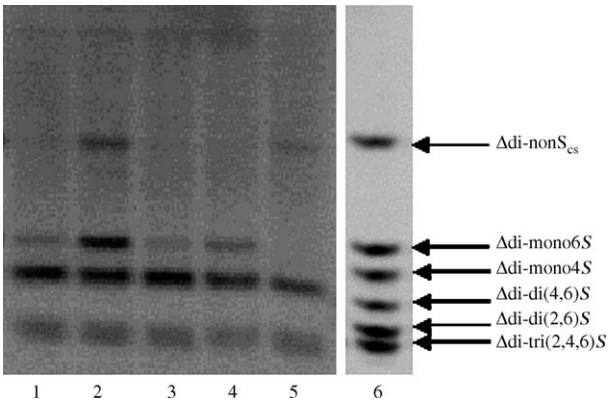


Fig. 2. PAGEFS of the 2-AMAC derivatized GAG disaccharides from different families of decorins; in lanes 1–5 were loaded 5 μl of the derivatized GAG disaccharides (1/20 of total volume, 2.5 nmol of total disaccharides) obtained from: lane 1, decorin D1 aorta (D1A); lane 2, decorin D1 tendon (D1T); lane 3, decorin D2 aorta (D2A); lane 4, decorin D2 tendon (D2T); lane 5, decorin D2 cornea (D2C); lane 6, mix of 40 pmol each of all the CS disaccharides mono-, di- and tri-sulphated.

Interestingly, the development of this technique allowed the characterization of the GAG chain of the decorins from different bovine tissues (Fig. 2). Even though decorins from different tissues have the same core proteins (data not shown), they showed different GAG structure when analysed by PAGEFS or HPLC analysis. Decorins purified from different bovine tissues and separated by standard ultracentrifuge technique in D1 and D2 subfamilies, could be quite different in GAG chain sulphation pattern (compare Fig. 2, lanes 2 and 4 for tendon samples). The subfamily D1T in tendon decorin contained more Δdi-nonS_{CS} and Δdi-mono6*S* than D2T subfamily, which contained mainly Δdi-mono4*S*. In decorin from aorta the sulphation pattern was different from that described in tendon, in fact D1A and D2A subfamilies showed similar sulphation pattern (compare Fig. 2, lanes 1 and 3) and Δdi-mono4*S* was the main disaccharide.

All data obtained by PAGEFS have been confirmed by HPLC equipped with fluorescence detector. Fig. 3 reports a typical HPLC separation, where in panel A standard disaccharide mixture including the Δdi-nonS_{HA} (peak 8) was analysed and panel B contains an example of disaccharide mixture from decorin GAG chain (D2T). In Table 1 we report all data obtained from GAG analysis, and the values reported are calculated from HPLC data, that are in good agreement with those obtained by PAGEFS. From the disaccharide analysis was evident that Δdi-di(2,6)*S* disaccharide was present in decorin from both aorta and tendon, even if slightly more abundant in the D1 fraction (Table 1). The tri- and di-sulphate disaccharides (Δdi-tri(2,4,6)*S* and Δdi-di(4,6)*S*) were also detectable, but in traces. Interestingly, decorin from cornea was mainly concentrated in D2 fraction (D2C). In this fraction Δdi-mono4*S* was predominant, with traces of Δdi-nonS_{CS} (Fig. 2, lane 5) and a particular low amount of Δdi-di(2,6)*S*.

Considering the core protein identity of all decorins, as previously described [16], the sulphation degree of GAG chains is critical for decorin binding properties to von Willebrand factor; in order to clarify more deeply those preliminary results, we carried out binding experiments using von Willebrand factor as ligand with decorins with very different sulphation pattern: decorin from bovine aorta (D2A) and decorin from bovine cornea (D2C). In Fig. 4, it is evident the big difference in binding properties between these two decorins: decorin from bovine aorta (D2A) bound with less efficiency to von Willebrand factor than decorin from bovine cornea (D2C).

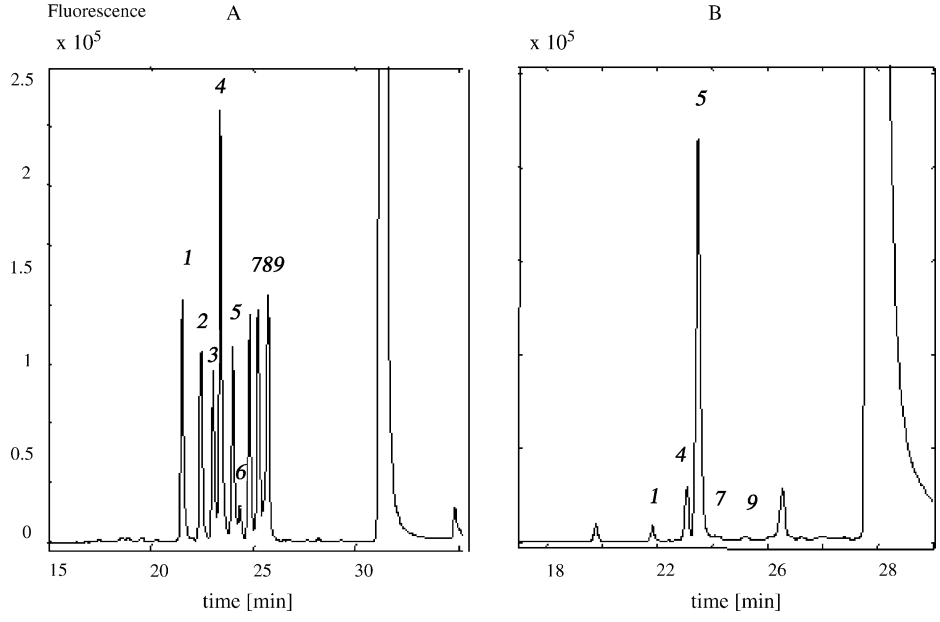
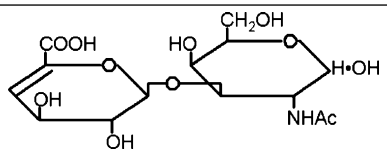
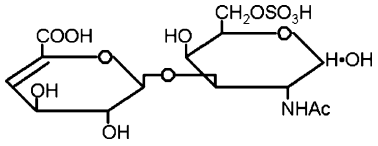
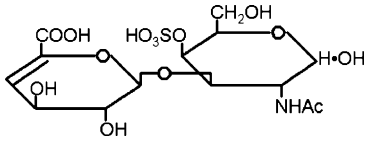
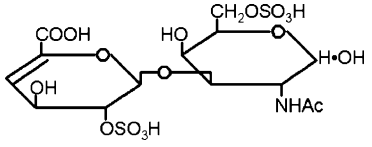
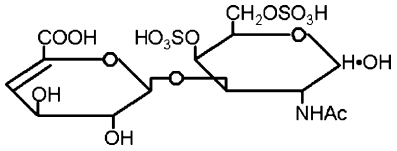
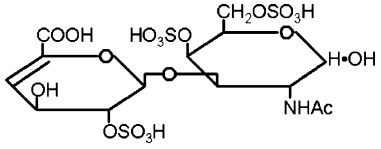


Fig. 3. HPLC chromatogram of: (A) mix of standard CS disaccharides 100 pmol each; (B) 10 μl of the derivatized GAG disaccharides (1/10 of total volume, 5 nmol of total disaccharides) of decorin after ultracentrifugation (sample D2T). Peaks correspond to: 1, Δdi-tri(2,4,6)*S*; 2, Δdi-di(2,4)*S*; 3, Δdi-di(4,6)*S*; 4, Δdi-di(2,6)*S*; 5, Δdi-mono4*S*; 6, Δdi-mono2*S*; 7, Δdi-mono6*S*; 8, Δdi-nonS_{HA}; 9, Δdi-nonS_{CS}.

Table 1
Distribution (%) of disaccharides present in the GAG of examined decorins measured by HPLC (see text for details)

Sample	Disaccharides	D1T	D1A	D2T	D2A	D2C
Δdi-nonS_{CS}		1.90	0.85	0.74	0.63	0.67
Δdi-mono6*S*		8.61	0	1.07	0.73	6.15
Δdi-mono4*S*		75.72	80.12	85.38	81.00	87.01
Δdi-di(2,6)*S*		11.86	16.07	10.21	14.47	3.39
Δdi-di(4,6)*S*		0	0.22	0	0.26	0
Δdi-tri(2,4,6)*S*		1.90	2.74	2.60	2.91	2.77

Analyzing the GAG composition in Table 1, it is noteworthy that decorin from aorta (D2A) contained GAG chains with higher amount of Δdi-di(2,6)*S* and lower amount of Δdi-mono6*S*, compared to decorin from cornea (D2C). On this basis, our data suggest that the position of sulphate group is the critical point in the interaction between GAG chains and von Willebrand factor.

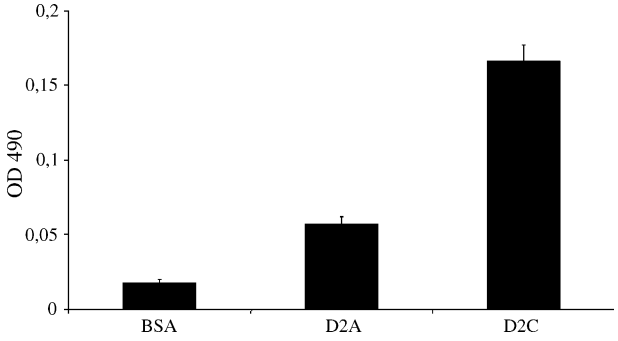


Fig. 4. von Willebrand factor (VWF) binding to decorins. Wells of microtiter plates were coated with: BSA; decorin from bovine aorta (D2A); decorin from bovine cornea (D2C). VWF binding to immobilized ligands was measured upon incubation a peroxidase-conjugated anti-VWF antibody revealed with a colorimetric reaction. Results are means of three different experiments and the S.D. is shown.

4. Conclusions

Decorin is one of the most studied proteoglycans in the last decades, not only for its presence and abundance in almost all tissues, for its role in regulating collagen fibril formation and the spatial arrangement of collagen fibres in the matrix [4], but also for its multiple binding ability with a lot of different ligands, among these cytokines as transforming growth factor-β [5], molecules of the epidermal growth factor receptor family [6,7], serpins [26] and recently, von Willebrand factor [16]. Decorin has also been proposed as tumor suppressor in cancer cells during malignant transformation, and overexpression of decorin in the tumor matrix, probably by myofibroblasts, has been postulated to represent a response of the host to invading cancer cells in an attempt to avert tumor spread [10–13].

Most of these studies were focused on the proteoglycan protein core, reporting gene sequences, splicing variants [14] and crystallographic properties [1,15], omitting the information deriving from the saccharidic chains, often not for defect

in availability but for the insufficient sensitivity of the used methods.

Moreover, the chemical nature and structural aspects of GAGs are now studied in more detail not only for their relevance in biological functions [16,26], but also for the methodological approaches now available, including among these PAGEFS recently proposed [17].

In this study, we present an approach developed from the already published PAGEFS method [17,25,28], useful to characterize the structure of chondroitin and dermatan GAG chains. These results were confirmed using HPLC as alternative method. PAGEFS was applied to decorin GAG chains and the encouraging results suggested that this approach can be an useful tool for studies on all proteoglycan containing galactosaminoglicuronan, as versican and aggrecan.

The modified PAGEFS consists of an electrophoretic procedure in which the salt buffer composition is Tris–HCl. Other studies reported that Tris–borate buffer is indicated for no sulphated and low sulphated disaccharide electrophoretic separation, but this buffer formulation was poorly efficient in sulphated disaccharides analysis [17,18]. The new condition we used can completely separate all the chondroitin/dermatan disaccharides, separating structural isomers as Δdi-mono- and Δdi-disulphate disaccharides differing only in the site sulphate position (e.g. Δdi-di(4,6)*S* and Δdi-di(2,6)*S* or Δdi-mono4*S* and Δdi-mono6*S*).

In our system all disaccharide bands were clearly separated both in single and in mixture preparation. As expected, the bands with higher sulphate content migrate faster whereas the bands with the same sulphate content showed slightly different migration and their separation is due, probably, for the different spatial geometry of the sulphate groups.

PAGEFS versus HPLC has various advantages: many samples/standards can be run in one gel that in HPLC, every run independently; the band intensity is independent of the nature of the disaccharides analysed as shown by O'Shea and co-workers [29] and Goubet et al. [30]; the sample amount is lower and the run of all samples requires less time.

Applying the PAGEFS method to decorins extracted from various bovine tissues, we were able to obtain specific information about the chemical structure of GAG chain and therefore we were able to describe the GAG sulphation pattern that is critical in the decorin binding to von Willebrand factor. The data reported in a previous work [16] indicated that the sulphation degree was an important requirement in discriminating decorins able to bind to von Willebrand factor. Interestingly, even though decorins from different tissues have the same core proteins, they showed different GAG structure when analysed by PAGEFS or HPLC. In fact, in our study, decorins purified from different bovine tissues and separated by standard ultracentrifuge technique in D1 and D2 subfamilies, showed remarkable differences in GAG chain sulphation (compare Fig. 2, lanes 2 and 4 for tendon samples). These data indicated that sulphation pattern of decorin is tissue specific, as shown in decorin from aorta and in decorin from tendon.

The possibility to assess carefully the Δdi-mono4*S* and Δdi-mono6*S* content in tissues is very important, considering that the ratio of these disaccharides changes during aging [19,20], and it could be used as a marker of matrix aging. The role of the sulphation pattern in decorin is therefore critical for its physiological functions and in particular the presence of high amount of Δdi-di(2,6)*S* disaccharide.

In conclusion, GAG chains of proteoglycans have not only a structural relevance, but this study demonstrated that GAG structure contributes to the biological activity of the proteoglycans. The improved PAGEFS method here described is therefore one of the simplest and fastest methods to address the GAG structure description.

Acknowledgements

The authors are in debt to Barbara Bartolini PhD and Mrs. Paola Moretto for their technical assistance during the experimental procedures. The authors are grateful to "Centro di Servizi Grandi Attrezzature per la Ricerca biomedica dell'Università dell'Insubria" for instruments availability. This work was sustained by funds from MIUR, PRIN to DV, PRIN to MET and CIB to AP.

References

[1] R.V. Iozzo, Annu. Rev. Biochem. 67 (1998) 609–652.
[2] A.M. Hocking, T. Shinomura, D.J. McQuillan, Matrix Biol. 17 (1998) 1–19.
[3] H. Kresse, H. Hausser, E. Schonherr, Experientia 49 (1993) 403–416.
[4] R.V. Iozzo, Crit. Rev. Biochem. Mol. Biol. 32 (1997) 141–174.
[5] Y. Yamaguchi, D.M. Mann, E. Ruoslahti, Nature 346 (1990) 281–284.
[6] D.K. Moscatello, M. Santra, D.M. Mann, D.J. McQuillan, A.J. Wong, R.V. Iozzo, J. Clin. Invest. 101 (1998) 406–412.
[7] M. Santra, T. Skorski, B. Calabretta, E.C. Lattime, R.V. Iozzo, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7016–7020.
[8] H. Hausser, H. Kresse, Biochem. J. 344 (1999) 827–835.
[9] J. Koninger, N.A. Giese, F.F. di Mola, P. Berberat, T. Giese, I. Esposito, M.G. Bachem, M.W. Buchler, H. Friess, Clin. Cancer Res. 10 (2004) 4776–4783.
[10] M.A. Nash, M.T. Deavers, R.S. Freedman, Clin. Cancer Res. 8 (2002) 1754–1760.
[11] M. Santra, I. Eichstetter, R.V. Iozzo, J. Biol. Chem. 275 (2000) 35153–35161.
[12] S. Troup, C. Njue, E.V. Kliewer, M. Parisien, C. Roskelley, S. Chakravarti, P.J. Roughley, L.C. Murphy, P.H.S. Watsson, Clin. Cancer Res. 9 (2003) 207–214.
[13] M. Santra, T. Skorski, B. Calabretta, E.C. Lattime, R.V. Iozzo, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7016–7020.
[14] W. Sheng, G. Wang, Y. Wang, J. Liang, J. Wen, P.S. Zheng, Y. Wu, V. Lee, J. Slingerland, D. Dumont, B.B. Yang, Mol. Biol. Cell. 16 (2005) 1330–1340.
[15] R.V. Iozzo, J. Biol. Chem. 274 (1999) 18843–18846.
[16] G.F. Guidetti, B. Bartolini, B. Bernardi, M.E. Tira, M.C. Berndt, C. Balduini, M. Torti, FEBS Lett. 574 (2004) 95–100.
[17] E.G. Karousou, M. Militsopoulou, G. Porta, G. De Luca, V.C. Hascall, A. Passi, Electrophoresis 25 (2004) 2919–2925.
[18] E.G. Karousou, G. Porta, G. De Luca, A. Passi, J. Pharm. Biomed. Anal. 34 (2004) 791–795.
[19] D.A. Carrino, P. Önnerfjord, J.D. Sandy, G. Cs-Szabo, P.G. Scott, J.M. Sorrell, D. Heinegård, A.I. Caplan, J. Biol. Chem. 278 (2003) 17566–17572.
[20] R. Albertini, G. De Luca, A. Passi, R. Moratti, P. Abuja, Arch. Biochem. Biophys. 365 (1999) 143–149.

[21] R. Tenni, M. Viola, F. Welser, P. Sini, C. Giudici, A. Rossi, M.E. Tira, Eur. J. Biochem. 269 (2002) 1428–1437.
[22] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[23] T. Bitter, H.M. Muir, Anal. Biochem. 4 (1962) 330–334.
[24] U.K. Laemmli, Nature 227 (1970) 680–685.
[25] A. Calabro, M. Benavides, M. Tammi, V.C. Hascall, R.J. Midura, Glycobiology 10 (2000) 273–281.
[26] A. Denti, P. Sini, E. Tira, C. Balduini, Thromb. Res. 79 (1995) 187–198.
[27] T.N. Seyfried, C.D. Blundell, A.J. Day, A. Almond, Glycobiology 15 (2005) 303–312.
[28] P. Jackson, Meth. Enzymol. 230 (1994) 250–265.
[29] M.K. Morell, M.S. Samuel, M.G. O'Shea, Electrophoresis 19 (1998) 2603–2611.
[30] F. Goubet, P. Jackson, M.J. Deery, P. Dupree, Anal. Biochem. 300 (2002) 53–68.