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Activity of chondroitin ABC lyase on dermatan sulfate partially degraded by cupric-ion-mediated free-radical treatment

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Abstract

Dermatan sulfate was extracted and purified from bovine intestinal mucosa, pig intestinal mucosa and pigskin. Small differences in M_r, charge density and constituent disaccharides were detected for the three purified natural dermatan sulfates. Bovine intestinal mucosa dermatan sulfate was depolymerized by a controlled free-radical process mediated by cupric ions in the presence of hydrogen peroxide. Different low-molecular-mass dermatan sulfate fractions were produced and analysed by high-performance size-exclusion chromatography and polyacrylamide gel electrophoresis. The results obtained by this last technique strongly support the hypothesis that the free-radical process proceeds essentially via the destruction of disaccharide units. The partial degradation of dermatan sulfates by cupric-ion-mediated free-radical treatment reduces or even eliminates the capacity of chondroitin ABC lyase to depolymerize these derivatives. This was confirmed by polyacrylamide gel electrophoresis and the time curves of enzymatic treatments evaluated by spectrophotometry.

1. Introduction[1]

Dermatan sulfate (chondroitin sulfate B) is a natural heteropolysaccharide that is very heterogeneous in terms of relative molecular mass (M_r), charge density, physico-chemical properties and biological activity [1]. The backbone of this sulfated glycosaminoglycan is mainly composed of L-iduronic acid (and D-glucuronic acid, to different extents depending on the

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[1] Abbrevations used: M_r = relative molecular mass; HPSEC = high-performance size-exclusion chromatography; LMM = low molecular mass; dp = degree of polymerization (e.g. dp = 2 for a disaccharide, 4 for a tetrasaccharide, etc.); ΔDi-0S = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose; ΔDi-4S = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose-4-sulfate; ΔDi-6S = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose-6-sulfate; ΔDi-2,6diS = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid 2-sulfate)-D-galactose-6-sulfate; ΔDi-2,4diS = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid 2-sulfate)-D-galactose-4- sulfate; ΔDi-4,6diS = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid)-D-galactose-4,6-disulfate; ΔDi-2,4,6triS = 2-acetamido-2-deoxy-3-O-(4-deoxy-α-L-*threo*-hex-4-enepyranosyluronic acid 2-sulfate)-D-galactose-4,6-disulfate.

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source) and N-acetyl-D-galactosamine. At position 2 of the iduronic acid and at position 6 of the N-acetyl-D-galactosamine [1], sulfate groups can be O-linked to the main structural disaccharide unit of sequence: [4)-O-(L-idopyranosyluronic acid)-β(1→3)-O-(2-acetamido-2-deoxy-D-galactopyranosyl 4-sulfate)-β(1→].

Currently, dermatan sulfate is under clinical development as a drug with anticoagulant and antithrombotic activity that essentially depends on binding and activation of heparin co-factor II [2,3]. Recently, the possibility that low- molecular-mass derivatives of dermatan sulfate (LMM-DSs) might be suitable for use as prophylactic antithrombotic drugs [4,5] has aroused great interest. LMM-DSs are generally prepared via controlled chemical or enzymatic depolymerization of commercial dermatan sulfate [1]. Whatever the preparation route, processing produces LMM derivatives having different structures and M_r distributions, resulting in a wide variety of products and therefore of biological activity and pharmacological properties.

Free-radical depolymerization mediated by cupric or ferrous ions is used to degrade natural polymers such as DNA, hyaluronic acid [6], heparin [7–9] and dermatan sulfate [10,11]. In the work reported in this paper, purified natural dermatan sulfate was depolymerized by a controlled free- radical process mediated by cupric ions. The different LMM-DSs obtained were analysed for peak molecular mass, using polyacrylamide gel electrophoresis as described by Rice et al. [12], and for their chondroitin ABC lyase degradability.

2. Experimental

2.1. Extraction and purification of dermatan sulfate

Bovine intestinal mucosa, pig intestinal mucosa and pigskin were each ground and treated separately with papain at 60°C for 24 h. After boiling for 30 min, the mixture in each case was brought to pH 9.0 by adding 2 *M* NaOH. After 24 h at 40°C, the product (brought to pH 6.0 with 2 *M* acetic acid) was centrifuged at 5000 *g* for 15 min, and the pellet washed twice with distilled water. Two volumes of acetone were added to the pooled supernatants, and the whole was then stored at + 4°C for 24 h. The precipitate was recovered by centrifugation at 5000 *g* for 15 min and dried at 60°C for 6 h. The dried precipitate was dissolved in distilled water by prolonged mixing. After centrifugation at 5000 *g* for 15 min, the supernatant was applied onto a column packed with Ecteola cellulosa (from Serva, Heidelberg, Germany) that had previously been washed with 1 *M* NaOH and 1 *M* HCl and equilibrated with 0.05 *M* ammonium acetate. After the resin had been washed with two volumes of 0.05 *M* ammonium acetate, dermatan sulfate was eluted with 0.4–1.8 *M* ammonium acetate, and two volumes of acetone were added to the eluate, which was then stored at + 4°C for 24 h. After centrifugation at 5000 *g* for 15 min, the pellet was dried at 60°C for 6 h. Dermatan sulfate was further purified by sequential precipitation with increasing volumes of acetone [13], by treatment with nitrous acid at low pH [14] and selective precipitation with copper acetate and acetone [15]. The dermatan sulfate copper salt was transformed into dermatan sulfate sodium salt on a Chelex 100 chelating resin (Code 142-2832 from Bio-Rad, Richmond, CA, USA). After elution, the pH of the percolate was adjusted to 6.0 by adding acetic acid, and then two volumes of acetone saturated with sodium acetate were added. Crude dermatan sulfate sodium salt was collected by precipitation with 1.0–1.5 volumes of acetone and dried.

2.2. Preparation of different free-radical-depolymerized dermatan sulfates

Bovine mucosa dermatan sulfate (5 g) and 0.2 g of copper acetate monohydrate (0.02 *M*) were dissolved in 50 ml of water in a reaction vessel. The temperature was kept at + 60°C and the pH adjusted to 7.5 by adding 1 *M* NaOH solution. A 9% hydrogen peroxide solution was added at a rate of 10 ml/h. The chemical depolymerization reaction was stopped by freezing at −20°C. After

the reaction, contaminating copper was removed from the product by the Chelex 100 chelating resin (Code 142-2832 from Bio-Rad). The pH of the percolate was adjusted to 6.0 by feeding in acetic acid, and then two volumes of acetone saturated with sodium acetate were added. The precipitate was collected by centrifugation and dried. The different LMM-DS samples were obtained by stopping the chemical depolymerization process at different times.

2.3. Time curves of chondroitin ABC lyase degradation of purified natural dermatan sulfate and free-radical-depolymerized dermatan sulfate

A total of 10 mg of dermatan sulfate samples (in 50 μl of 50 m*M* Tris-HCl buffer, pH 8.0) were incubated with 125 mU (in 50 μl of 50 m*M* Tris-HCl buffer, pH 8.0) of chondroitinase ABC (E.C. 4.2.2.7) (Seikagaku Kogyo, Tokyo, Japan) in 100 μl of 50 m*M* Tris-HCl buffer, pH 8.0 at 37°C. At each measurement interval, 10-μl samples were drawn. After boiling for 1 min, 5 μl of each sample was stored at −20°C until required for polyacrylamide gel electrophoresis analysis. The remaining 5 μl was tested for spectrophotometric absorbance (Spectrophotometer Model V-550 from Jasco, Tokyo, Japan) at 232 nm in 500 μl of 30 m*M* HCl.

2.4. Polyacrylamide gel electrophoresis

Electrophoresis was performed on a Bio-Rad Protean IIxi vertical-slab-gel unit connected to a Pharmacia LKB (Pharmacia, Uppsala, Sweden) 2219 Multitemp II thermostatic circulator. Power was supplied by an LKB 2197 generator. The gel was prepared essentially as reported by Rice et al. [12]. The resolving gel and lower buffer chamber contained 0.1 *M* boric acid–0.1 *M* Tris–0.01 *M* disodium EDTA buffer, pH 8.3. The stacking gel was prepared in the same buffer adjusted to pH 6.3 with HCl. The upper buffer chamber contained 0.2 *M* Tris–1.25 *M* glycine hydrochloride, pH 8.3. The gels were poured vertically, using a gel-pouring stand, between glass plates (20 × 16 cm) separated by 1.0 mm spacers. Gradient gels were poured by adding 20 ml of 12% total acrylamide (acrylamide + bisacrylamide) solution to the front chamber of the gradient apparatus and 20 ml of 25% total acrylamide solution to the back chamber. A 5-ml portion of stacking-gel solution containing 5% total acrylamide was applied to the top of the resolving gel. Dermatan sulfate samples (5–10 μl) were combined with 20 μl of 50% (w/v) sucrose solution containing 0.1% (w/v) bromophenol blue (Sigma, St. Louis, MO, USA). Electrophoresis was performed at 50 mA (400–500 V) for 2.5–3.0 h at 5°C. Gels were removed from the glass plates and stained for 30 min in 0.08% (w/v) Azure A (Sigma, St. Louis, MO, USA) in water. The stain was subsequently rinsed out.

Gel scans were performed by a densitometer consisting of a Macintosh IIsi computer interfaced with a Microtek Color Scanner from Microtek International (Hsinchu, Taiwan). The IMAGE processing and analysis program, Version 1.41 from Jet Propulsion Lab. (NASA, FL, USA) was used to perform densitometric analysis of polyacrylamide gel electrophoretic bands.

2.5. Determination of relative molecular mass

The HPLC equipment was from Jasco (Tokyo, Japan). It comprised a pump, Model 880 PU, a system controller, Model 801 SC, a ternary gradient unit Model 880-02, a Rheodyne injector equipped with a 100-μl loop, and a UV detector, Model 875 UV. The mobile phase was composed of 125 m*M* Na_2SO_4 and 2 m*M* NaH_2PO_4 adjusted to pH 6.0 with 0.1 *M* NaOH. The flow-rate was 0.9 ml/min with a back pressure of 25 kg/cm^2. The columns Protein Pak 125 (300 × 7.8 mm; particle size: 10 μm; molecular mass ranges: natural globular from 2000 to 80 000 and random coil from 1000 to 30 000; Code 84601 from Waters, Milford, MA, USA) and 300 (300 × 7.5 mm; particle size: 10 μm; molecular mass ranges: natural globular from 10 000 to 400 000 and random coil from 2000 to 150 000; Code T72711 from Waters) were assembled in series. Different dermatan sulfate samples were solubilized in the mobile phase at a concen-

tration of 10 mg/ml; 10 μl (100 μg) was injected into the HPLC unit.

The peak M_r of dermatan sulfate samples was determined by a calibration curve plotted with glycosaminoglycan standards, as reported elsewhere [16]. The third grade polynomial regression was calculated by a Macintosh computer program.

2.6. Quantification of constituent disaccharides by cleavage with chondroitinase ABC

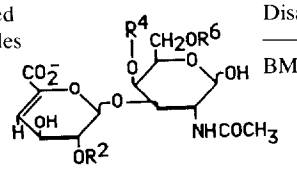
Natural dermatan sulfate samples [100 μg (10 mg/ml in water)] were incubated with 50 mU of chondroitinase ABC (E.C. 4.2.2.7.) in 50 m*M* Tris-HCl buffer, pH 8.0. The reactions were stopped after 3 h of incubation at 37°C by 1 min boiling.

Constituent disaccharides were determined by strong-anion exchange (SAX)-HPLC. HPLC equipment was by Jasco as described in Section 2.5. The column used was 5-μm Spherisorb SAX, 250 × 4.6 mm (trimethylammoniopropyl groups $Si\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}N^+(CH_3)_3$ in Cl^- form), from Phase Separations, Deeside Industrial Park, Deeside Clwyd, UK. Isocratic separation was performed for 0 to 5 min with 0.10 *M* NaCl pH 4.00; linear gradient separation was carried out for 5 to 60 min from 100% 0.10 *M* NaCl (brought to pH 4.0 with 1 *M* HCl) to 100% 1.20 *M* NaCl (brought to pH 4.0 with 1 *M* HCl). The flow-rate was 1.4 ml/min. The UV wavelength was set at 232 nm. Enzymatically degraded dermatan sulfate samples (20 μg) were injected. Unsaturated disaccharides of dermatan sulfate were separated using the standards and retention times recommended by Yoshida et al. [17].

3. Results

Table 1 reports the physico-chemical properties and disaccharide composition of the dermatan sulfates purified from bovine mucosa, pig mucosa and pigskin. Possible contaminant glycosaminoglycans (chondroitin sulfate A and C, slow-moving and fast-moving components of heparin) in the dermatan sulfate preparations were determined by agarose gel electrophoresis, as reported elsewhere [18]. No other glycosaminoglycans were detected in the natural dermatan sulfate samples by this technique. Polyacrylamide gel electrophoresis of the bovine mucosa dermatan sulfate which had undergone prolonged treatment with chondroitin ABC lyase shows material resistant to the exhaustive enzymatic treatment (Fig. 5, see below). This material was not further characterized or analysed. Small differences in M_r, charge density and constituent disaccharides were detected between the three natural dermatan sulfates, with

Table 1
Physico-chemical properties and percentage of unsaturated non-sulfated and sulfated disaccharides derived from the polysaccharide chains of natural dermatan sulfates from different sources by chondroitin ABC lyase cleavage

Unsaturated disaccharides				Disaccharides (%)[a]		
				BM	PM	PS
	R^2	R^4	R^6			
ΔDi-0S	H	H	H	1.2	2.5	3.2
ΔDi-6S	H	H	SO_3^-	3.0	5.6	2.5
ΔDi-4S	H	SO_3^-	H	85.6	78.9	80.8
ΔDi-2,6diS	SO_3^-	H	SO_3^-	0.3	1.1	0.1
ΔDi-4,6diS	H	SO_3^-	SO_3^-	1.5	4.3	6.4
ΔDi-2,4diS	SO_3^-	SO_3^-	H	8.3	7.2	6.8
ΔDi-2,4,6triS	SO_3^-	SO_3^-	SO_3^-	0.1	0.4	0.2
M_r (× 1000)				28.2	31.6	43.8
SO_3^-/COO^- [b]				1.09	1.11	1.11

BM = bovine mucosa; PM = pig mucosa; PS = pigskin.

[a] The quantity of each disaccharide identified was determined using purified standards and reported as weight percentage. Under the experimental conditions used, cleavage of natural dermatan sulfate samples with chondroitinase ABC produces 100% disaccharides (also evaluated by polyacrylamide gel electrophoresis) and no chondroitin sulfate oligosaccharides resistant to the treatment of lyase.

[b] The sulfate-to-carboxyl ratio was determined by enzymatic degradation after HPLC separation of the constituent disaccharides. The ratio was calculated taking into account the percentage and the presence of carboxyl and sulfate groups for each disaccharide.

pigskin dermatan sulfate showing a higher M_r (see also Fig. 1) and percentage of disulfated disaccharides. The sulfate-to-carboxyl ratio is similar for the three samples.

Fig. 1 illustrates the HPSEC profiles of the three natural dermatan sulfates and of three samples obtained by free-radical depolymerization of bovine mucosa dermatan sulfate after different times (30, 60 and 105 min: peak M_r 14 520, 7360 and 1530, respectively). Under our experimental conditions, the M_r of bovine mucosa dermatan sulfate was reduced by about one half after 30 min and by about one quarter after 60 min. These results are consistent with a process of chemical depolymerization of glycosaminoglycans in which the M_r decreases with time according to an exponential-like function [10,16].

The photograph in Fig. 2 shows polyacrylamide gel electrophoresis of the three natural dermatan sulfates (PS: pigskin; PM: pig mucosa; and BM: bovine mucosa) and of free-radical-degraded dermatan sulfate samples obtained by chemical depolymerization of bovine mucosa dermatan sulfate after different reaction times. The peak M_rs of free-radical-depolymerized dermatan sulfate samples evaluated by HPSEC are reported in the figure legend. Natural dermatan sulfate and free-radical-degraded samples are compared with a sample of dermatan sulfate (from bovine mucosa) partially depolymerized by enzymatic treatment with chondroitin ABC lyase (L). This photograph illustrates the separation of saccharide species with different degrees of polymerization (up to dp28 as detected by densitometric scanning, see Fig. 3). The electrophoretic migrations of oligosaccharide species detected in free-radical-degraded bovine mucosa dermatan sulfate samples at different times are the same (as evaluated by comparing their electrophoretic mobility) as those of oligosaccharide fractions obtained by enzymatic treatment with chondroitin ABC lyase of natural bovine mucosa dermatan sulfate.

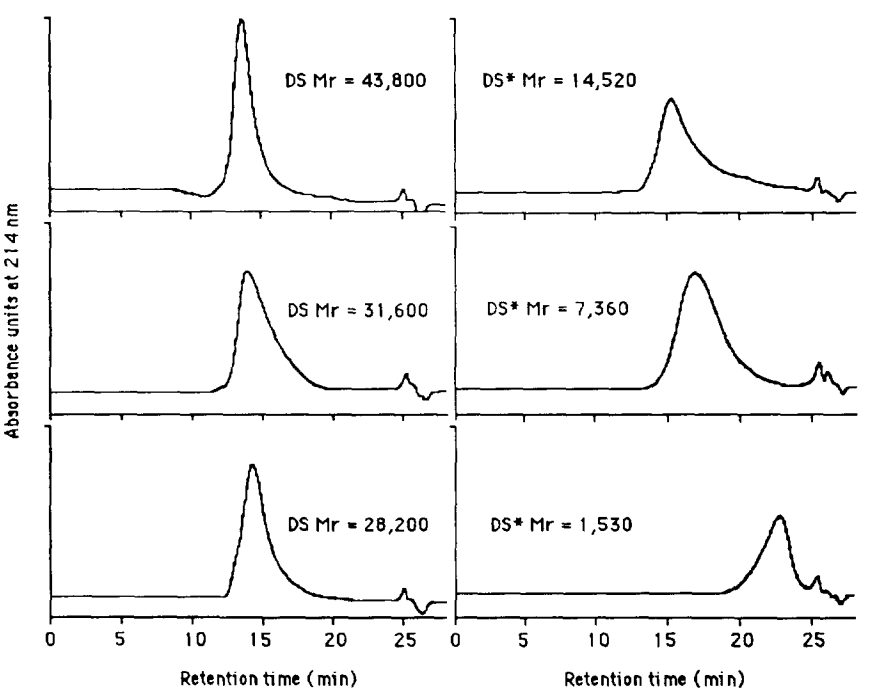


Fig. 1. High-performance size-exclusion chromatographic profiles of three natural dermatan sulfate samples (DS) and three free-radical-depolymerized samples (DS*) obtained by chemical treatment of bovine mucosa dermatan sulfate after different times (30 min, peak M_r = 14 520; 60 min, peak M_r = 7360; 105 min, peak M_r = 1530).

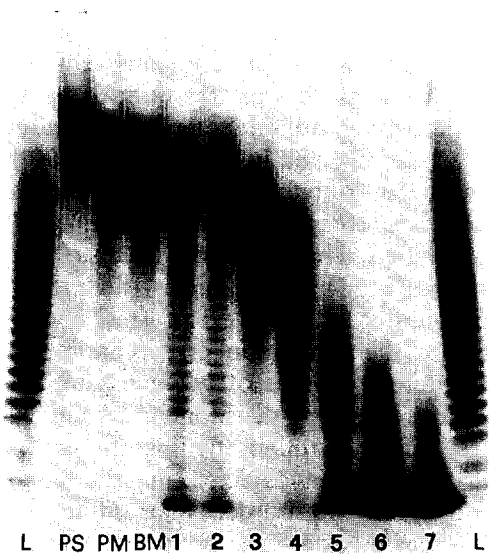


Fig. 2. Polyacrylamide gel electrophoresis of three natural dermatan sulfate samples (pigskin: PS; pig mucosa: PM; bovine mucosa: BM) and free-radical-depolymerized bovine mucosa dermatan sulfate samples obtained after different reaction times (1: 15 min, peak M_r = 19 110; 2: 30 min, peak M_r = 14 520; 3: 45 min, peak M_r = 11 560; 4: 60 min, peak M_r = 7360; 5: 75 min, peak M_r = 4350; 6: 90 min, peak M_r = 2580; 7: 105 min, peak M_r = 1530) compared with dermatan sulfate (from bovine mucosa) partially depolymerized by chondroitin ABC lyase (L).

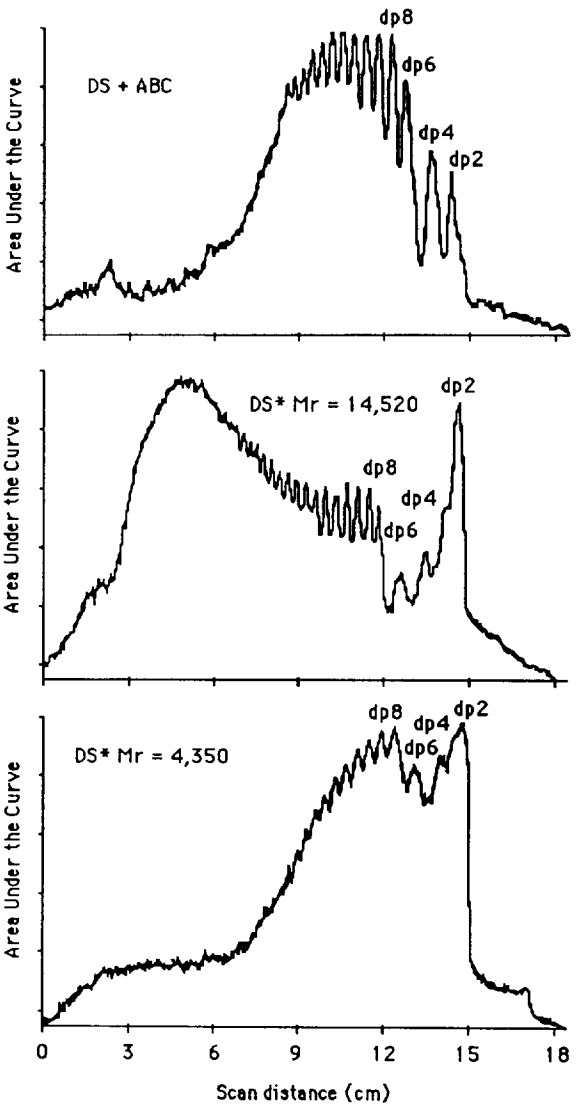


Fig. 3. Densitometric scannings after separation by polyacrylamide gel electrophoresis of bovine mucosa dermatan sulfate treated with chondroitin ABC lyase (DS + ABC), and free-radical-depolymerized bovine mucosa dermatan sulfate samples obtained after 30 min (peak M_r = 14 520, DS* 14 520) and 75 min (peak M_r = 4350, DS* 4350). The enzymatic degradation of bovine mucosa dermatan sulfate by chondroitin ABC lyase (DS + ABC) was stopped after 2 min by 1 min boiling, and densitometric scanning was performed on the polysaccharide species at a lower degree of polymerization (dp = up to 28).

Further evidence of this is provided in Fig. 3, which compares the densitometric scannings (after separation by polyacrylamide gel electrophoresis) of bovine mucosa dermatan sulfate treated with chondroitin ABC lyase and of bovine mucosa dermatan sulfate samples obtained by free-radical depolymerization after 30 min (peak M_r = 14 520) and 75 min (peak M_r = 4350).

Fig. 4 shows the time curves of chondroitin ABC lyase degradation of the three natural dermatan sulfate samples and of the free-radical-depolymerized bovine mucosa dermatan sulfate samples at different times measured by spectrophotometric analysis at 232 nm. As illustrated in the figure, the three natural dermatan sulfates were degraded extensively by chondroitinase ABC, and the enzymatic reactions reach equilibrium after about 4–5 h under the experimental conditions adopted. In contrast, after 15 min of chemical treatment (M_r = 19 110) the free-radical bovine mucosa dermatan sulfate is degraded by chondroitinase ABC only to a limited extent,

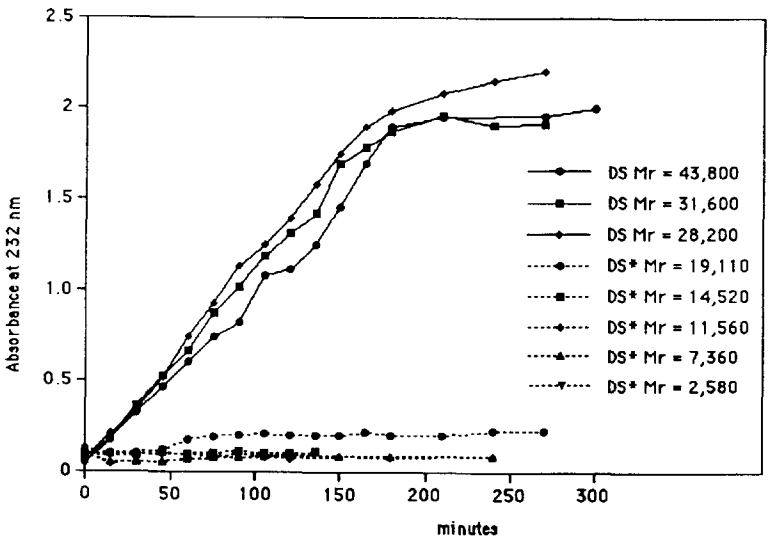


Fig. 4. Time curves, determined by spectrophotometry at 232 nm, of chondroitin ABC lyase degradation of the three natural dermatan sulfates (DS) and of five free-radical-depolymerized bovine mucosa dermatan sulfates (DS*) obtained by stopping the chemical reaction at different times.

whilst the other free-radical dermatan sulfate samples are not depolymerized by lyase at all, even after 4 h of treatment. This is confirmed in Figs. 5 and 6. In fact, natural dermatan sulfate is completely degraded by chondroitin ABC lyase after 90 min (Fig. 5), producing disaccharides (last electrophoretic band in each lane). Bovine mucosa dermatan sulfate treated with enzyme for 2 min has a large number of high-M_r polysaccharide chains, with oligosaccharides showing decreasing M_r up to disaccharides. Samples degraded with lyase for 30 and 60 min (lanes B and C in Fig. 5) contain larger amounts of low-M_r oligosaccharide species.

Fig. 5 also shows that bovine mucosa dermatan sulfate degraded with chondroitinase ABC for 90 min (lane D) and longer contains material resistant to the exhaustive enzymatic treatment. This material is a contaminant from bovine mucosa dermatan sulfate preparation that is not detected by the agarose gel electrophoresis used to evaluate the purity of tissue extracts. In fact, as previously reported [18], this technique can detect only about 4% of contaminant glycosaminoglycans in a preparation of heparin or dermatan sulfate. Complete degradation of dermatan sulfate and its further separation by polyacrylamide gel electrophoresis shows contaminant material in low concentration in the bovine mucosa dermatan sulfate preparation. On the other hand, this material is not appreciably degraded by chondroitinase ABC, and there is no evidence of formation of oligosaccharide species with differences in disaccharide units typical of the eliminase mechanism of polysaccharide lyases [19]. Furthermore, this material is present at the same concentration in dermatan sulfate samples degraded with chondroitin ABC lyase after 90 min and after 345 min, confirming the incapacity of this lyase to degrade it. Even though this contaminant material was not further characterized and analysed, we can exclude the presence of glucosaminoglycans (heparin and/or heparan sulfate) due to the treatment with nitrous acid at low pH [14].

The free-radical-depolymerized dermatan sulfate sample (M_r = 14 520) obtained after 30 min of chemical processing was treated with chondroitin ABC lyase for different times and analysed by polyacrylamide gel electrophoresis (Fig. 6). This technique confirms that samples of dermatan sulfate obtained by free-radical de-

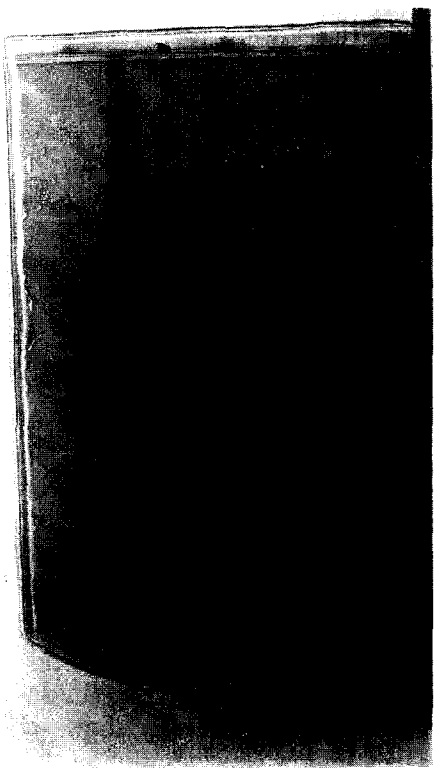


Fig. 5. Polyacrylamide gel electrophoresis time curves of chondroitin ABC lyase degradation of bovine mucosa dermatan sulfate. A: 2 min; B: 30 min; C: 60 min; D: 90 min; E: 120 min; F: 180 min; G: 345 min.

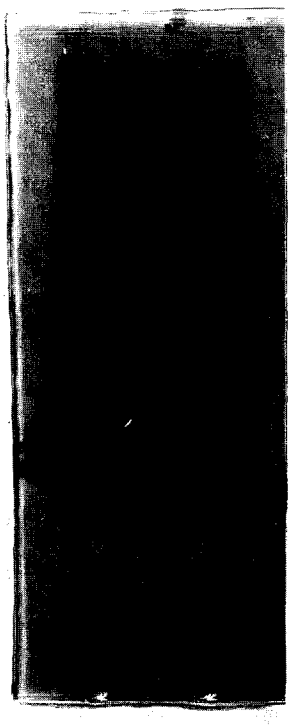


Fig. 6. Polyacrylamide gel electrophoresis time curves of chondroitin ABC lyase degradation of free-radical-depolymerized bovine mucosa dermatan sulfate (M_r peak = 14 520). A: 2 min; B: 30 min; C: 60 min; D: 90 min; E: 120 min.

polymerization are not degraded by lyase at all, or are only degraded by lyase to a very limited extent, even after 120 min of enzymatic reaction (compare lanes A and E in Fig. 6).

4. Discussion

Hydroxyl radicals formed by the reaction of ferrous or cupric ions with hydrogen peroxide or molecular oxygen are used as active agents in the oxidation of various organic polymers. The addition of ascorbic acid enhances the activity of this chemical reaction, and chelation of the ions with EDTA also increases the rate of oxidation. Glycosaminoglycans, hyaluronic acid, heparin, dermatan sulfate and chondroitin sulfate are natural polymers used in pharmacology. LMM glycosaminoglycans are employed as derivatives with different properties, such as better pharmacokinetics [20], reduced side effects, new forms of biological activity, and various routes of administration. These LMM derivatives are prepared by chemically or enzymatically controlled depolymerization reactions, and free-radical degradation mediated by ions is one of the more commonly used chemical processes due to its low cost in large-scale preparation.

Hyaluronic acid was degraded by oxidative reductive depolymerization mediated by ferrous ions under an oxygen atmosphere [6]. This process proceeds essentially by random destruction of monosaccharide units, followed by secondary hydrolytic cleavage of the resulting unstable glycosidic substituents. Heparin was depolymerized by this chemical approach, and in this case too, the reaction proceeds by destruc-

tion of monosaccharide units except for iduronic acid 2-sulfate residues [8]. Substitution of cupric ions for ferrous ions results in a more selective modification of heparin [7], and the free-radical attack appears to occur adjacent to some residues of iduronic acid 2-sulfate. With the cupric reagent, dermatan sulfate was reported to undergo more extensive degradation than heparin [7].

In particular, under the experimental conditions used here, i.e. large amounts of cupric ions, high temperature (60°C) and controlled pH, dermatan sulfate is rapidly degraded after about 2 h to produce a derivative with an M_r of about 1500. Polyacrylamide gel electrophoresis analysis strongly supports the hypothesis that the free-radical reaction degrades dermatan sulfate at the disaccharide units. This was also observed for chondroitin sulfate depolymerized under the same experimental conditions (data not shown). On the other hand, we cannot exclude the possibility that, after exhaustive treatment, the reaction proceeds by destruction of monosaccharide units.

Free-radical-depolymerized dermatan sulfates are not degraded by chondroitin ABC lyase, or are only degraded to a very small extent. This means that these derivatives are more resistant to the action of enzymes, in particular those involved in catabolic processes. We can suppose that glycosaminoglycan derivatives (in this case, dermatan sulfate derivatives) produced by free-radical degradation used for pharmacological purposes might be more resistant to the enzymatic processes involved in the removal of these substances by the human body. On the other hand, this implies greater persistence of these derivatives in biological compartments, such as blood, tissues and organs, and different pharmacokinetics as compared to the natural products.

The results of this study indicate that free-radical glycosaminoglycan derivatives have different properties from those of glycosaminoglycan purified from tissues and that they should not be considered "natural products".

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