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Capillary electrophoresis to measure sulfoesterase activity on chondroitin sulfate and heparin derived disaccharides

Azra Pervin, Kenan Gu & Robert J. Linhardt

Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242, USA

Capillary electrophoresis was used to assay sulfoesterase activity on sulfated disaccharides derived from chondroitin sulfate, dermatan sulfate and heparin. The three sulfoesterases studied were chondro-4-*O*-sulfatase (EC 3.1.6.9) and chondro-6-*O*-sulfatase (EC 3.1.6.10) from *Proteus vulgaris* and heparo-2-*O*-sulfatase from *Flavobacterium heparinum*. Capillary electrophoresis was used to analyse sulfated disaccharide before and after sulfoesterase treatment and a change in migration time was indicative of the presence of sulfoesterase activity. This assay was used both on purified sulfoesterases and on minor sulfoesterase contaminants present in other enzyme preparations. The high sensitivity of capillary electrophoresis permits the elimination of ^{35}S-radiolabeled substrates normally required to assay sulfoesterases. The high resolution of capillary electrophoresis allows the use of this assay on impure enzyme preparations containing high protein concentrations.

Keywords: 2-, 4- or 6-*O*-sulfoesterases; chondroitin/dermatan sulfate or heparin derived disaccharides; assay; capillary electrophoresis.

Introduction

Capillary electrophoresis (CE) is a powerful, high resolution analytical technique for separation, characterization and quantitation of minute amount of analytes. Detection of attomole and zeptomole amounts have been reported with the use of laser induced fluorescent detectors (Gassman *et al.*, 1985; Burton *et al.*, 1986). CE is a simple, rapid and automatable method of analysis (Jorgenson, 1986; Compton & Brownlee, 1988; Gorden *et al.*, 1988; Ewing *et al.*, 1989) and has been applied to separation of diverse species including polypeptides, glycoproteins and acidic polysaccharides (Jorgenson & Lukacs, 1983; Lauer & McManigill, 1986; Desai *et al.*, 1992; Honda *et al.*, 1992).

Separation by electrophoresis is based on difference in mobilities of charged species. While electrically neutral species have no electrophoretic mobility their movement through the capillary to the negative electrode still takes place as the result of electro-osmotic flow. Similarly, negatively charged species migrate towards the negative electrode in a direction opposite to their electrophoretic mobility, as the result of electro-osmotic flow. Acidic oligosaccharide mobility depends on the charge to size ratio, sample concentration, the buffer system (its pH and ionic strength), the voltage applied, the temperature inside the capillary and length and diameter of the capillary (Ampofo *et al.*, 1991; Desai *et al*, 1992).

CE has been used for the analysis of negatively charged disaccharides prepared from heparin, heparan sulfate (Ampofo *et al.*, 1991), chondroitin and dermatan sulfate, (Al-Hakim & Linhardt, 1991) as well as analysis of higher oligosaccharides derived from heparin (Desai *et al.*, 1992). These studies suggest the application of CE to assay enzymatic activity using these acidic disaccharides as substrates. The growth of the *Flavobacterium heparinum* (also called *Cytophaga heparina* (Christensen, 1980) on heparin or chondroitin sulfate is accompanied by the production of enzymes capable of degrading heparin and chondroitin sulfates. These enzymes include sulfoesterases that act on specific, sulfated disaccharides. Sulfoesterases have been difficult to assay, generally requiring the preparation of ^{35}S-labeled substrates or the use of tedious HPLC methods. Problems associated with rapidly assaying sulfoesterase activity have made the purification of these enzymes problematic. Thus, very few sulfoesterases are commercially available and none has been purified to homogeneity. The present communication describes the applications of capillary electrophoresis to assay of 2-, 4-, or 6-*O*-sulfatase action on disaccharides prepared from chondroitin sulfate and heparin.

Materials and methods

Materials

Chondro-4-*O*-sulfatase (EC 3.1.6.9 from *Proteus vulgaris*) and chondro-6-sulfatase (EC 3.1.6.10 from *P. vulgaris*) were from Seikagaku America Inc., Rockville, MD. A heparo-2-*O*-sulfatase from *F. heparinum* that acts on disaccharides derived from heparin or heparan sulfate was the generous gift of Dr Yoshida of Seikagaku, Tokyo. Chondroitin AC lyase, heparin lyase I, heparin lyase II and heparin lyase III were prepared in our laboratory from *F. heparinum* (Gu *et al.*, 1992; Lohse & Linhardt,1992). These enzymes are also commercially available from Seikagaku and Sigma Chemical, St Louis, MO. The heparin lyase II used in this study had not been subjected to gel permeation

Correspondence: R.J. Linhardt, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242, USA
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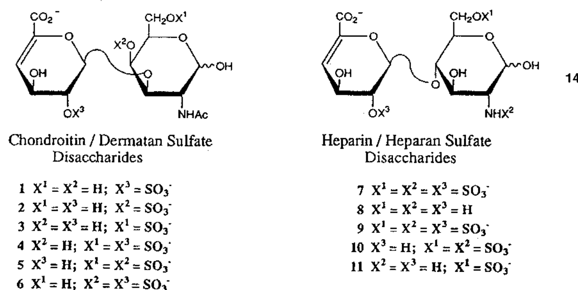

HPLC, the final step required for preparation of a homogenous protein (Lohse & Linhardt, 1992). Chondroitin sulfate and dermatan sulfate derived disaccharide standards (see structures) were obtained from Seikagaku America and heparin and heparan sulfate derived disaccharide standards from Grampian Enzymes, Aberdeen, Scotland or from Sigma. Bovine serum albumin was from Sigma. All other chemicals were reagent grade. Capillary electrophoresis was performed using a Dionex Capillary Electrophoresis System (Sunnyvale, CA).

Methods

Capillary electrophoresis Separation and analysis were carried out in a fused silica (externally coated except where the tube passed through the detector) capillary tube (75 µm i.d., 375 µm o.d. and 68 cm long) from Dionex. The capillary tube was washed extensively with 0.1 M phosphoric acid, 0.5 M sodium hydroxide and deionized, distilled water, then filled with operating buffer (10 mM sodium borate and 50 mM sodium dodecylsulfate (SDS) at a pH 8.8) inserted into the machine. The sample was injected by gravity injection 45 mm, injection time was 25 ms (corresponding to 15 nl). CE was performed at constant voltage of approximately 10 or 20 kV as described in the text. Detection was by ultraviolet absorbance at 232 nm.

Assay for chondro-6-O-sulfatase activity

Chondroitin sulfate or dermatan sulfate derived disaccharide (**3**), ΔUAp-(1→3)-β-D-GalNpAc6S (ΔUAp is 4-deoxy-α-L-*threo*-hex-enopyranosyluronic acid; GalNp is 2-deoxy-2-aminogalactopyranose; S is sulfate; Ac is acetate), 2 nmol in 30 µl of 0.4 M Tris-hydrochloride buffer at pH 7.9 containing 0.4 M sodium acetate and 0.1% bovine serum albumin (BSA) was treated at 37°C for 25 min with 8 mU of 6-*O*-sulfatase enzyme. The reaction was terminated by heating at 100°C for 1 min. The 6-*O*-sulfatase activity was analysed by capillary electrophoresis.

Disulfated and trisulfated chondroitin sulfate or dermatan sulfate derived disaccharides containing sulfate group at the 6-position including ΔUAp-2S-(1→3)-β-D-GalNpAc6S (**4**), ΔUAp-(1→3)-β-D-GalNAc4S,6S (**5**) and ΔUAp2S-(1→3)-β-D-GalNpAc4S,6S (**7**) were also used as substrates for sulfatase activity. Disulfated disaccharide substrate (2 nmol) was incubated in 8 mU of chondro-6-*O*-sulfatase using 30 µl of 25 mM imidazole buffer at pH 7 containing 25 mM sodium chloride, 5 mM magnesium chloride, 1 mM calcium chloride and 0.05% (w/v) BSA. The hydrolysis was performed at 25°C and terminated after 1 h by heating to 100°C for 1 min.

Assay for chondro-4-O-sulfatase

Chondroitin sulfate or dermatan sulfate derived disaccharide (**2**) ΔUAp-(1→3)-β-D-GalNpAc4S (2 nmol) was treated with 10 mU of chondro-4-*O*-sulfatase for 25 min at 37°C in 30 µl Tris-hydrochloride buffer described for chondro-6-*O*-sulfatase with 10 mU of chondro-4-*O*-sulfatase. The reaction was terminated after 25 min by heating at 100°C. Disulfated and trisulfated chondroitin sulfate or dermatan sulfate derived disaccharides, ΔUAp-(1→3)-β-D-GalNpAc4S,6S (**5**) ΔUAp2S-(1→3)-β-D-GalNpAc4S (**6**) and ΔUAp2S-(1→3)-β-D-GalNpAc4S,6S (**7**) also served as substrates for the chondro-4-*O*-sulfatase assay (performed in the same sample buffer used for disulfated substrates in the chondro-6-*O*-sulfatase assay).

Assay for heparo-2-O-sulfatase

Disaccharide **9** derived from heparin ΔUAp2S(–1→4)-β-D-GlcNp2S,6S (GlcNp is 2-deoxy-2-aminoglucopyranose) (2 nmol) was treated at 25°C for 1 h in 30 µl of 50 mM boric acid and 10 mM sodium borate buffer (as described for chondro-6-*O*-sulfatase) with approximately 10 mU of heparo-2-*O*-sulfatase. The digestion was stopped by heating 1 min at 100°C.

Assaying sulfatase contamination in purified enzyme preparations

(i) Sulfatase activity in chondroitin AC lyase: Purified chondroitin AC lyase (35 mU) in 25 µl of Tris-hydrochloride buffer (described for chondro-6-*O*-sulfatase) was added to 1 mg of ΔUAp-(1→3)-β-D-GalNpAc4S (**2**) or ΔUAp-(1→3)-β-D-GalNpAc6S (**3**). After a 24 h incubation at 37°C the enzyme was thermally inactivated at 100°C for 1 min. The presence of sulfatase activity was determined by CE from the disappearance of monosulfated substrate and appearance of unsulfated product.

(ii) Sulfatase activity in hepain lyase I, II and III: Heparin lyase I, II and III (35 mU) were dissolved in 30 µl of 25 mM imidazole buffer at pH 7.0, containing 5 mM magnesium chloride, 25 mM sodium chloride, 1 mM calcium chloride and 0.05% (w/v) BSA. The reaction was incubated overnight at 25°C in the presence of ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**). The presence of 2-*O*-sulfatase activity in heparin lyase II was measured by the disappearance of a peak corresponding to ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**) and appearance of ΔUAp-(1→4)-β-D-GlcNp2S,6S (**10**).

(iii) Assaying sulfatase during enzyme purification: During the purification of heparin lyase II (Lohse & Linhardt, 1992) fractions were assayed for sulfatase contamination by incubating overnight at 25°C in presence of trisulfated disaccharide, ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**).

Results

Assay for chondro-6-O-sulfatase activity

Chondro-6-*O*-sulfatase from *P. vulgaris* (Seikagaku literature, 1991) can catalyze the hydrolysis of the sulfate group at position 6 of *N*-acetyl-galactosamine-

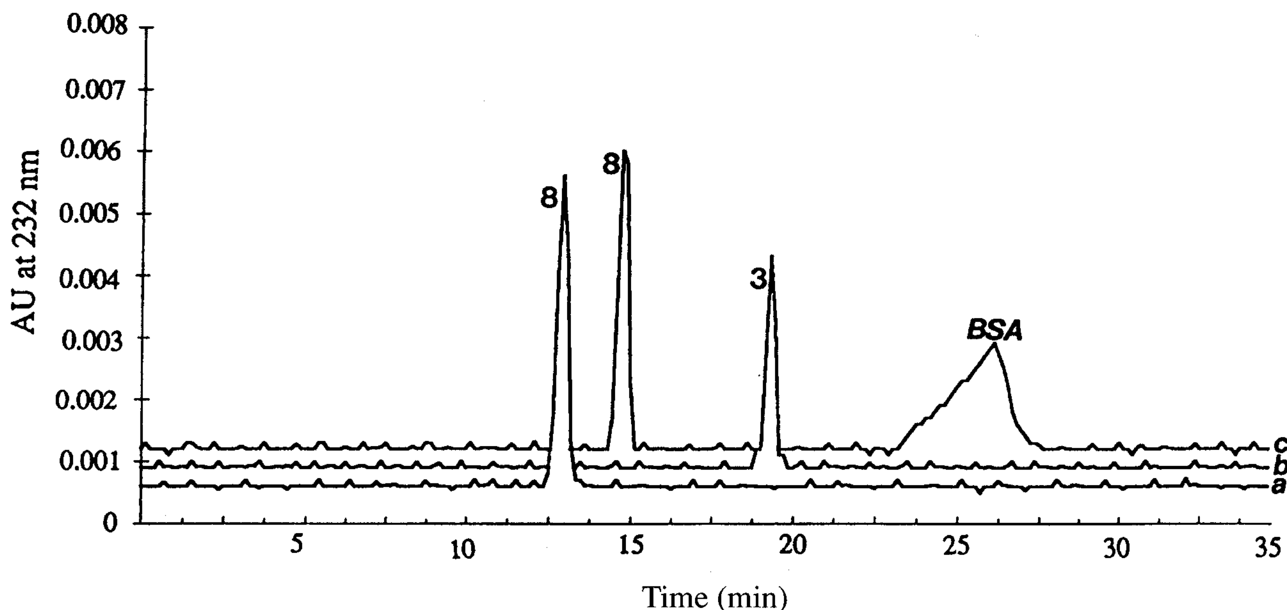

Figure 1 Capillary electropherograms of disaccharide standards **8** and **3** (a and b) and disaccharide **3** treated with chondro-6-*O*-sulfatase (c)

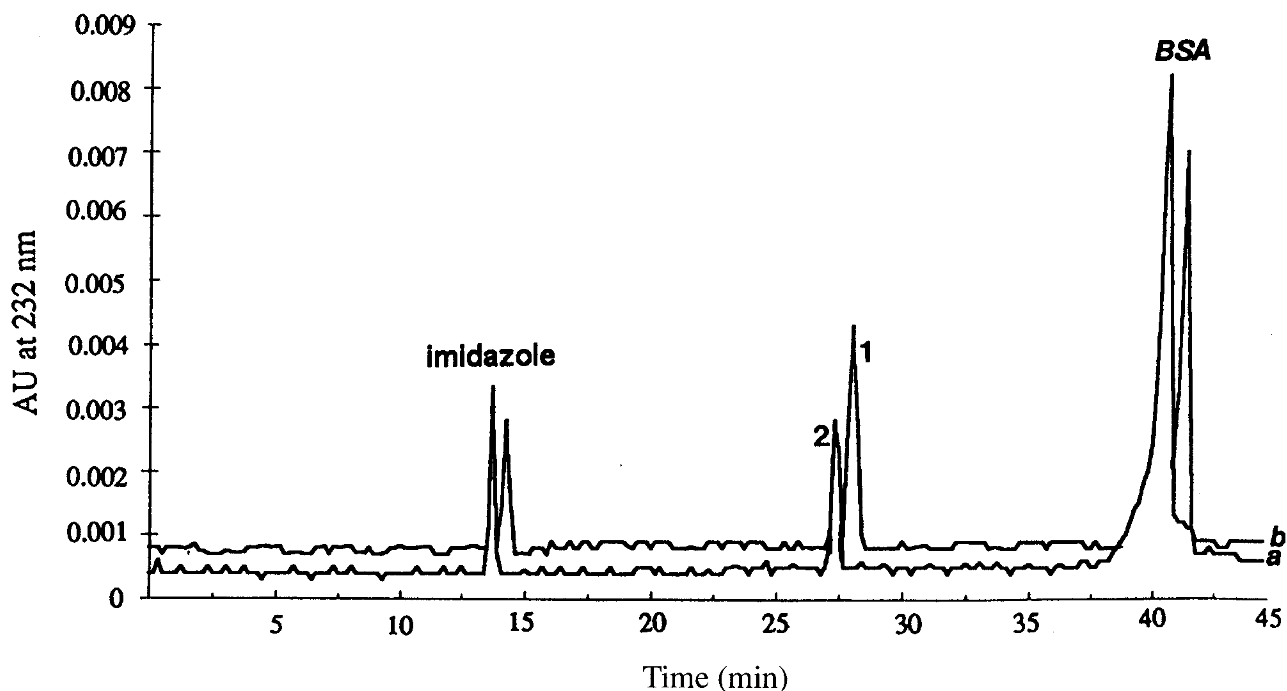

Figure 2 Capillary electropherograms of disaccharides **1** and **2** (a and b) formed on treatment of **4** and **5**, respectively, with chondro-6-*O*-sulfatase in imidizole buffer containing BSA. The peaks at 14 and 42 min are due to imidazole and BSA, respectively

6-sulfate residue in saturated or unsaturated monò (6-*O*) sulfated disaccharides. Such disaccharides can be obtained from chondroitin sulfates by digestion with either chondroitin sulfate lyases (chondroitin ABC, A, B, and AC lyases) or hyaluronidase (a hydrolase) (Yamagato *et al.*, 1968). Chondro-6-*O*-sulfatase reportedly can also hydrolyze the sulfate group at 6-position from 2,6 and 4,6 disulfated unsaturated disaccharides derived from chondroitin or dermatan sulfate.

Disaccharide (**3**), ΔUAp-(1→3)-β-D-GlcNpAc6S, (2 nmol) was treated with 8 mU of chondro-6-*O*-sulfatase and specifically hydrolyzed into ΔUAp-(1→3)-β-D-GlcNpAc (**8**). Disaccharides **3** and **8** were prepared in distilled water (μg μl^{-1}) as standards. Both enzyme treated disaccharide and standard disaccharides were analysed by CE in buffer containing 10 mM sodium borate and 50 mM SDS at pH 8.8, at a constant voltage of 10 kV and detected by UV absorbance at 232 nm (Figure 1). The migration of the substrate and product were 19 and 13 min, respectively. The electropherogram of enzyme treated disaccharide **3** resulted in a peak that correlated to migration time of

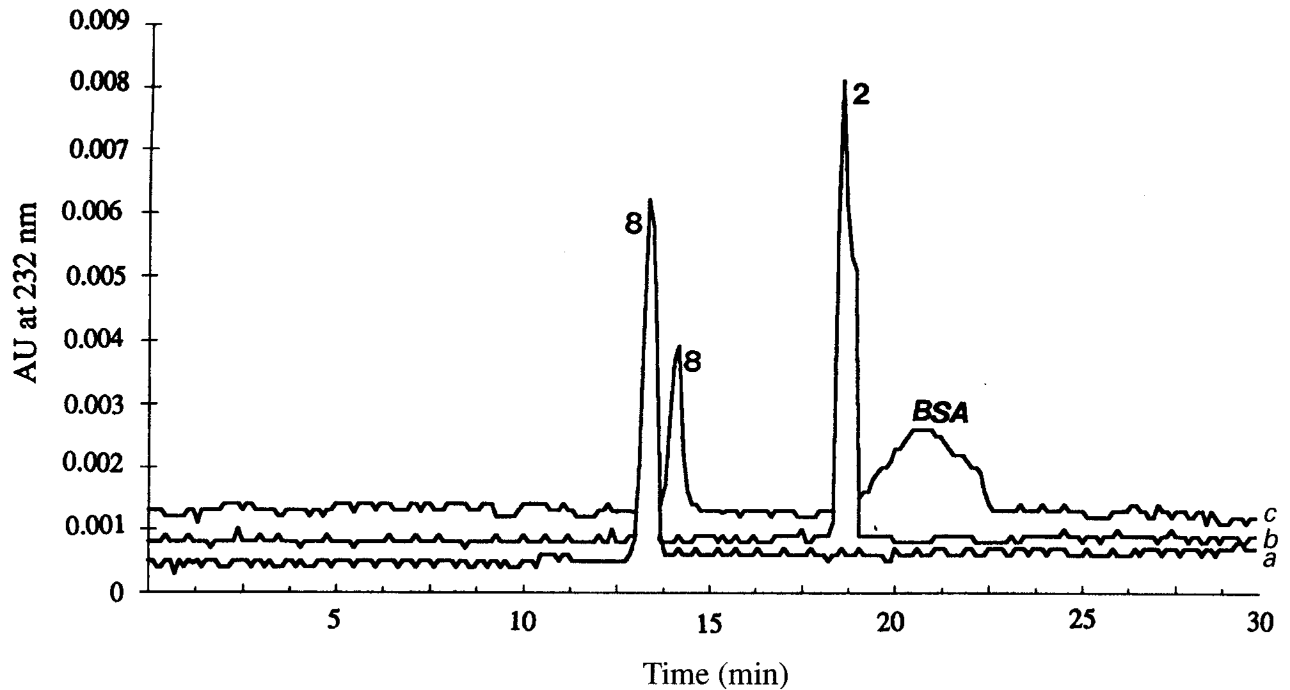

Figure 3 Capillary electropherograms of disaccharide standards **8** and **2** (a and b) and disaccharide **2** treated with chondro-4-*O*-sulfatase (c)

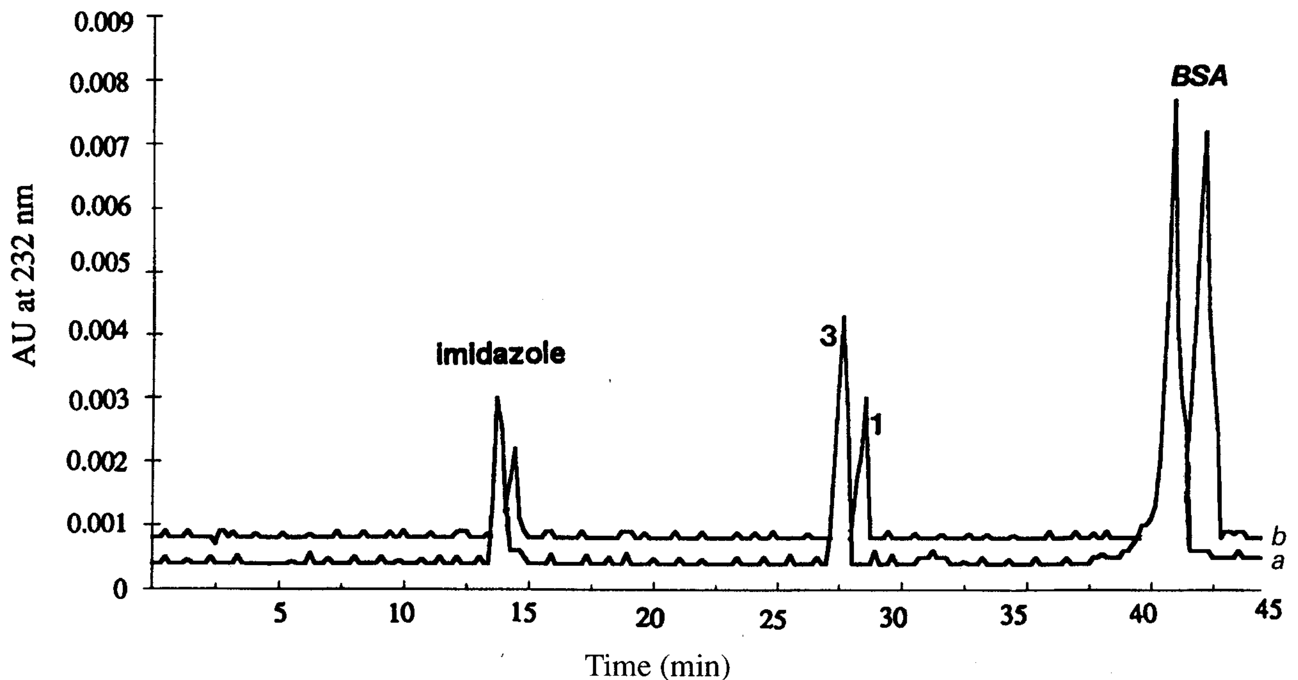

Figure 4 Capillary electropherograms of disaccharide **3** and **1** (a and b) formed on treatment of **5** and **6**, respectively, with chondro-4-*O*-sulfatase in imidizole buffer containing BSA. The peaks at 14 and 42 min are due to imidazole and BSA, respectively

disaccharide **8** demonstrating that 6-*O*-desulfation had occurred (Figure 1). Coinjection of disaccharide **8** with the product of chondro-6-*O*-sulfatase acting on **3** gave a single symmetrical peak (not shown).

Chondro-6-*O*-sulfatase also reportedly catalyzes the desulfation of 6-*O*-sulfate group from disulfated and trisulfated chondroitin or dermatan sulfate derived disaccharides. Disulfated disaccharides ΔUAp2S-(1→3)-β-D-GalNpAc-6S (**4**) and ΔUA-(1→3)-β-D-GalNpAc-4S, 6S (**5**) were each treated with 8 mU of chondro-6-*O*-sulfatase in Tris-hydrochloride buffer. Electrophoresis following treatment of **4** or **5** with chondro-6-*O*-sulfatase did not show any change in migration times (electropherograms no shown). The same disulfated disaccharides **4** and **5** were then each treated with 8 mU of chondro-6-*O*-sulfatase in

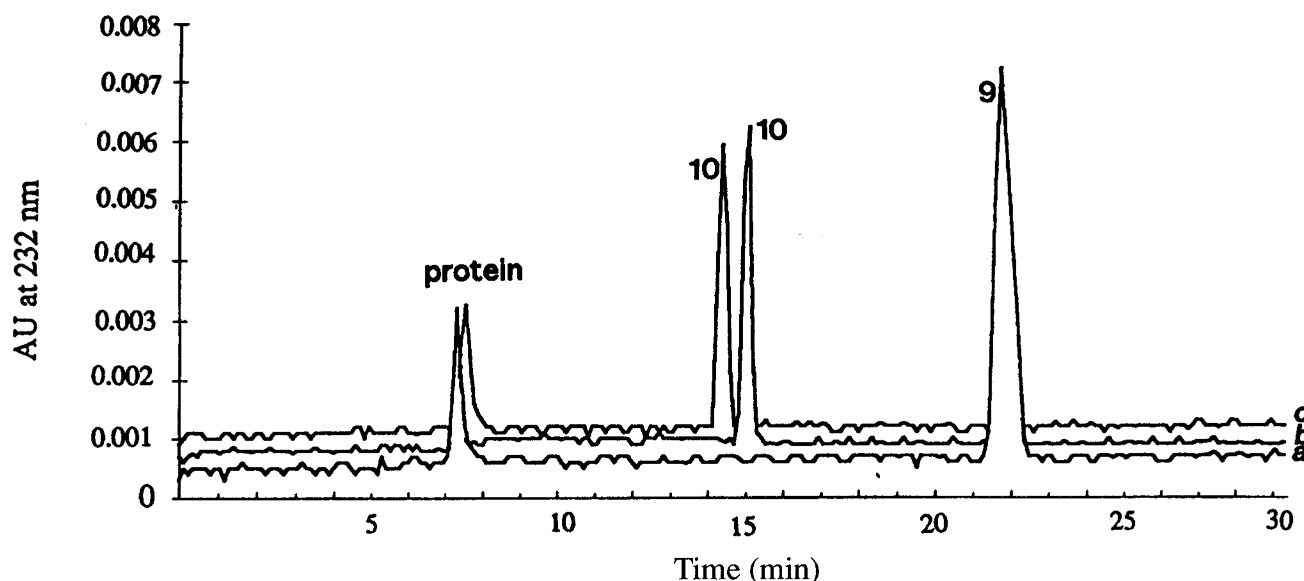

Figure 5 Capillary electropherograms of disaccharide standards **9** and **10** (a and b) and disaccharide **9** treated with heparo-2-*O*-sulfatase (c)

imidazole buffer (Bruce *et al.*, 1985a,b). As a result of chondro-6-*O*-sulfatase action, these disulfated disaccharides, were converted into monosulfated disaccharides ΔUAp2S-(1→3)-β-D-GalNpAc, (**1**) and ΔUAp-(1→3)-β-D-GalNpAc-4S, (**2**) respectively (Figure 2). On CE at 10 kV, the disulfated, disaccharide substrates **4** and **5** migrated at 34 and 37 min, respectively. Their monosulfated disaccharide products, **1** and **2** migrated at 28.2 and 27.9 min respectively. Again, the hydrolyzed products migrated faster than the more highly charged substrates. These results confirm the known activity of chondro-6-*O*-sulfatase. These results show that 0.4 M Tris-hydrochloride buffer is not effective for de-sulfation of disulfated disaccharides by chondro-6-*O*-sulfatase enzyme as reported in Seikagaku's product literature. The use of imidazole buffer overcomes this problem.

Assay for Chondro-4-O-sulfatase activity

Chondro-4-*O*-sulfatase from *P. vulgaris* (Seikagaku literature, 1991) hydrolyzes the sulfate group at position 4 of the *N*-acetyl-galactosamine residue in saturated or unsaturated disaccharides derived from chondroitin or dermatan sulfates (again prepared using lyase or hydrolase). While this enzyme reportedly does not act on the disulfated or trisulfated disaccharides, tetrasaccharides containing 4-*O*-sulfate, derived from chondroitin sulfate are reportedly substrates (Seno *et al.*, 1974).

Disaccharide, ΔUAp-(1→3)-β-D-GlcNpAc4S, (**2**) (2 nmol) was treated with 10 mU of chondro-4-*O*-sulfatase in Tris-hydrochloride buffer and converted to ΔUAp-(1→3)-β-D-GalNpAc- (**8**) as demonstrated by the change in mobility of product on CE analysis at 10 kV (Figure 3). The migration times of **8** and **2** were 19 and 13 min, respectively.

Disulfated and trisulfated chondroitin or dermatan sulfate derived disaccharides (1 mg ml^{-1}): ΔUAp-(1→3)-β-D-GalNpAc-4S,6S (**5**), ΔUAp2S-(1→3)-β-D-GalNpAc-4S (**6**) and ΔUAp2S-(1→3)-β-D-GalNpAc-4S,6S (**7**) were treated with 8 mU of chondro-4-*O*-sulfatase in imidazole buffer. CE analysis at 10 kV confirmed these substrates were converted to products (Figure 4). Disaccharide substrates **5**, **6**, and **7** migrated at 37, 38 and 55 min. Disaccharide products **3**, **1**, and **4** (not shown) migrated at 28.0, 28.4 and 38.6 min (Figure 4), respectively.

Assay for heparo-2-O-sulfatase

Heparo-2-*O*-sulfatase from *F. heparinum* catalyzes the hydrolysis of sulfate group at position 2 from the uronic acid residue of unsaturated disaccharides derived from heparin. These disaccharides are prepared by digestion of heparin with heparin lyase I and heparin lyase II (Linhardt *et al.*, 1990). Disaccharide **9**, ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (2 nmol) was treated with approximately 10 mU of heparo-2-*O*-sulfatase in 30 μl of 50 mM boric acid and 10 mM sodium borate buffer. Heparo-2-*O*-sulfatase catalyzed the removal of the sulfate group from position 2 of the unsaturated uronic acid residue (ΔUAp2S) converting disaccharide ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**) to ΔUAp-(1→4)-β-D-GlcNp2S,6S (**10**) as demonstrated by the change in the mobility of the product on CE (Figure 5). Disaccharide substrate **9** migrated at 22 min while disaccharide product **10** migrated at 14 min correlating with the migration time of the standard heparin derived disaccharide ΔUAp-(1→4)-β-D-GlcNp2S,6S (**10**).

Assaying sulfatase contamination in purified enzyme preparations or during enzyme purification

The approach described for assay purified sulfatase can also be used to detect 2-, 4-, or 6*O*-sulfatase contaminations in purified chondroitin AC and B lyase, glycuronidase and heparin lyases I, II and III (Gu et al., 1992; Lohse & Linhardt, 1992).

Monosulfated disaccharides: ΔUAp2S-(1→4)-β-D-GlcNpAc (**1**), ΔUAp-(1→3)-β-D-GalNpAc4S (**2**), and

ΔUAp-(1→3)-β-D-GalNpAc6S (**3**) were used as substrates to assay the presence of chondro-2-*O*-sulfatase, chondro-4-*O*-sulfatase and chondro-6-*O*-sulfatase activities, respectively whereas heparin derived disaccharide ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**) was used as a substrate for heparo-2-*O*-sulfatase assay. Each substrate (1 mg ml^{-1}) was treated with 35 mU of enzyme (with suspected sulfatase contamination) in 30 μl of 25 mM imidazole buffer at pH 7.0 containing 25 mM sodium chloride, 5 mM magnesium chloride, 1 mM calcium chloride and 0.05% (w/v) BSA. After incubation at 25°C for 60 min the reaction was terminated by heating in a boiling water bath for 1 min. On capillary electrophoresis a decrease in the peak intensity or disappearance of the peak for disaccharide substrate and the appearance of a disaccharide product having reduced sulfation was indicative that the enzyme was contaminated with sulfatase activity.

CE analysis of purified chondroitin AC lyase, heparin lyase I, heparin lyase II and heparin lyase III showed that only a partially purified heparin lyase II preparation contained sulfatase contamination. This contamination was indicated by the conversion of ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**) to ΔUAp-(1→4)-β-D-GlcNp2S,6S (**10**) (data not shown). Protein present in the assay mixture migrates as a single broad peak in the CE buffer containing SDS (as in Figures 2 and 4). This protein peak at 42 min (10 kV) does not interfere with the determination of disaccharides. Thus, the sulfatase assay described can be used to assay minor amounts of sulfatase present as a contaminant in a purified enzyme preparation.

Discussion

Korn and Payza first determined the presence of sulfamidase and *O*-sulfatase activity in *F. heparinum* (Korn & Payza, 1956). A number of laboratories have partially purified 3-*O*-, 2-*O*-, and 6-*O*-sulfatases and sulfamidase from *F. heparinum* (Linker & Sampson, 1960; McLean *et al.*, 1984; Bruce *et al.*, 1985 a,b).

A variety of bacteria reportedly produce *O*-sulfatase enzymes (Dietrich *et al.*, 1973; Bruce *et al.*, 1985 a,b). In reviewing the various *O*-sulfatase assays described in literature (Linker & Sampson, 1960; McLean *et al.*, 1984; Bruce *et al.*, 1985; Bruce *et al.*, 1987) it became apparent that virtually all of these assays required radioactive substrate. The synthesis and purification of these radioactive substrates relied on multiple steps. In sulfatase assays, these substrates are treated with enzyme to release inorganic sulfate, which is typically precipitated using barium chloride. The sulfatase activity is then determined by measuring radioactivity of the precipitated sulfate salt. The carbohydrate products were often also purified by chromatography and their structures determined. Thus, the standard methods of *O*-sulfatase assay are complicated, time consuming and tedious.

Dietrich *et al.* first described an assay for heparo-2-*O*-sulfatase using a trisulfated disaccharide substrate ΔUAp2S-(1→4)-β-D-GlcNp2S,6S (**9**). Paper chromatography was used to separate the ^{35}S-disulfated product from inorganic ^{35}S-sulfate. Heparo-2-*O*-sulfatase was shown to act specifically on disaccharides.

Ototani *et al.* also developed heparo-2-*O*-sulfatase assay using disulfated disaccharide, ΔUAp2S-(1→4)-β-D-GlcNp2S, and measuring the monosulfated product colorimetrically using a thiobarbituric acid assay specific for the ΔUAp residue present in the de-*O*-sulfated ΔUAp2S-(1→4)-β-D-GlcNp2S product (Ototani *et al.*, 1981).

Chondro-*O*-sulfatase assays (Bruce *et al.*, 1985 a,b, 1987) required the preparation of three ^{35}S-labeled monosaccharides and one ^{14}C-labeled substrate. The preparation of radioactive substrates from their corresponding monosaccharides: GlcNp-6^{35}S, GlcNp2S,6^{35}S and GlcNpAc-6^{35}S were carried out in pyridine/[^{35}S] sulfur trioxide complex with chloro-[^{35}S]-sulfonic acid. After several steps, the radioactive monosaccharides were purified by passing through Dowex AG-50W-X8 (H^+ form) and radioactivity was measured. These substrates were incubated in presence of enzyme 6-*O*-sulfatase at 25°C. After desulfation the reaction was terminated by trichloroacetic acid and the inorganic sulfate product was precipitated with barium chloride and counted.

Ideally, sulfatase assays should be simple, easy to perform in a short amount of time, reproducible and should not require the synthesis of radioactive substrates. Naturally occurring acidic polysaccharides: heparin, heparan sulfate, chondroitin sulfates and dermatan sulfate are highly rich in sulfate groups at the 2-, 4-, and 6-positions. Disaccharides prepared from chondroitin sulfate, dermatan sulfate and heparin using polysaccharide lyases are commercially available. Chondro-4-*O*-sulfatase and chondro-6-*O*-sulfatase are commercially available enzymes that selectively catalyze the desulfation of 4- and 6-*O*-sulfate groups from disaccharides prepared from chondroitin sulfates and dermatan sulfate. Heparo-2-*O*-sulfatase selectively catalyzes the desulfation of the 2-*O*-sulfate group from disaccharides prepared from heparin or heparan sulfate.

Chondroitin and dermatan sulfate derived disaccharides, ΔUAp-(1→3)-β-D-GalNpAc-4S (**2**) and ΔUAp-(1→3)-β-D-GalNpAc-6S (**3**) are useful as substrates for the chondro-4-*O*-sulfatase, chondro-6-*O*-sulfatase, respectively. Heparin derived disaccharide ΔUAp-2S-(1→4)-β-D-GlcNp2S,6S (**9**) is useful as a substrate for heparo-2-*O*-sulfatase. These substrates were treated with *O*-sulfatase enzymes in 0.4 M Tris-hydrochloride containing 0.4 M sodium acetate buffer and 0.1% (w/v) BSA at pH 7.9. This buffer was suitable for desulfating monosulfated disaccharides with chondro-4- and 6-*O*-sulfatase. Imidizole buffer was required for the hydrolysis of disulfated disaccharides by chondro-4- or 6-*O*-sulfatase.

Our group had already reported the separation of eight chondroitin sulfate and dermatan sulfate derived disaccharides and either heparin and heparan sulfate derived disaccharides (Al-Hakim & Linhardt, 1991; Ampofo *et al.*, 1991) using CE in 10 mM sodium borate and 50 mM sodium dodecylsulfate at pH 8.8 under constant voltage of 20 kV. Taking advantages of migration times of these disaccharides (charge to size ratio correlate to migration time), very simple and reproducible 2-, 4- and 6-*O*-sulfatase assays have been developed.

The 2-, 4-, or 6-*O*-sulfatases catalyzed the removal of sulfate groups from commercially available

disaccharide substrates. As a result of this desulfation, the charge to size ratio for each of these disaccharides decreased, changing their mobility. Both substrate and product contained a ΔUA or ΔUA2S residue at their non-reducing end, resulting in an absorbance at 232 nm (ϵ_M = 5000–6000 cm^{-1}) and permitting their detection. We have previously reported a sensitivity of detection of 500 amol for these disaccharides separated by CE using ultraviolet detection (Al-Hakim & Linhardt, 1991).

The presence of SDS in the buffer used removes the interference of BSA added as a stabilizer to the enzyme buffer and permits the convenient and sensitive detection of sulfatase activity even when it is only a minor contaminant in another enzyme preparation. This rapid and sensitive assay should greatly assist the future purification of new sulfatase activities.

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