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Separation of Glycosaminoglycan-Derived Oligosaccharides by Capillary Electrophoresis Using Reverse Polarity

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A comparative study on compositional analysis of two sets of eight unsaturated disaccharide standards derived from heparin/heparan sulfate and chondroitin/dermatan sulfate was carried out using capillary electrophoresis performed in both normal and reverse polarity modes. While these heparin/heparan sulfate disaccharides (S. A. Ampofo, H. M. Wang, and R. J. Linhardt (1991) *Anal. Biochem.* 199, 249–255) and chondroitin/dermatan sulfate disaccharides (A. Al-Hakim and R. J. Linhardt (1991) *Anal. Biochem.* 195, 68–73) have previously been fractionated using normal polarity capillary electrophoresis, multiple buffer systems and conditions were required to separate certain disaccharide isomers and these separations often resulted in poor peak symmetry and significant tailing. This paper demonstrates that reverse polarity capillary electrophoresis completely resolves disaccharide mixtures into all components using a single buffer, 20 mM phosphoric acid–sodium phosphate at pH 3.48. This improved resolution is due primarily to an increase in the sharpness of peaks and improved peak symmetry. Separation of heparin-derived oligosaccharides, ranging from disaccharide to hexasaccharide, had also previously been reported using normal polarity capillary electrophoresis (U. R. Desai, H. M. Wang, S. A. Ampofo, and R. J. Linhardt (1993) *Anal. Biochem.* 213, 120–127). This paper now demonstrates the separation of 13 heparin-derived oligosaccharides of sizes ranging from disaccharide to tetradecasaccharide using both reverse and normal polarities. An enzymatic digestion of bovine lung heparin containing many of these larger oligosaccharides was also compared in both normal and reverse polarity modes. Mixtures containing oligosaccharides primarily differing in size (number of saccharide units) were better resolved using normal polarity. © 1994 Academic Press, Inc.

Capillary electrophoresis (CE)[2] is a technique capable of the rapid, high-resolution separation of analytes requiring an extremely small amount of sample in a narrow (50–100 μm, i.d.) fused-silica capillary of 0.5–1.5 m in length (1–4). CE is performed at high voltage in normal polarity (positive mode) or reverse polarity (negative mode). In normal polarity the sample is applied at the anode and detected at the cathode; negative analytes are prevented from migrating under electrophoresis toward the anode by the bulk flow of solvent toward cathode resulting from electroendoosmosis, caused by the charged silanol residues of the capillary column (5). In reverse polarity, the silanol residues on the capillary column are uncharged because of the low pH. Since the polarity of the electrode is reversed, the sample is applied at the cathode and migrates under electrophoresis toward the anode. The resolution achieved by CE under a given set of conditions is dependent mainly on the charge, mass, and molecular mobility of the analytes present. Detection is typically by ultraviolet absorbence or fluorescence emission (1–4).

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[2] Abbreviations used: CE, capillary electrophoresis; LMW, low molecular weight; ΔUAp, 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid; IdoA, idopyranosyluronic acid; S, sulfate; GlcNp, 2-deoxy-2-aminoglucopyranose; GlcNpAc, 2-deoxy-2-acetamidoglucopyranose; SAX, strong anion exchange; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; NMR, nuclear magnetic resonance; MWCO, molecular weight cutoff; UA, uronic acid; RPIP, reversed phase ion-pairing.

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Heparin, heparan sulfate, dermatan sulfate, and chondroitin sulfates are highly charged, linear acidic polysaccharides called glycosaminoglycans. Heparin is widely used as a clinical anticoagulant (6), while heparan sulfate influences cell growth development and differentiation (7,8). Heparin and heparan sulfate are composed of alternating 1 → 4 linked glucosamine and uronic acid residues (9), while chondroitin sulfate and dermatan sulfate are composed of alternating 1 → 3 linked *N*-acetylgalactosamine and uronic acid residues (10). The polysaccharide lyases are bacterial enzymes that convert glycosaminoglycans to unsaturated disaccharide and oligosaccharide products (11–15). There are three polysaccharide lyases that act on heparin and heparan sulfate, heparin lyase I (EC 4.2.2.7), heparin lyase II (no EC assigned), and heparin lyase III (EC 4.2.2.8) (16). Complete digestion of heparin and heparan sulfate with the appropriate polysaccharide lyase affords primarily disaccharide products, while partial digestion markedly increases the percentage of higher oligosaccharides. Chondroitin sulfate and dermatan sulfate can be nearly completely converted to disaccharide products using chondroitin ABC lyase (14,15,17,18).

Our laboratory previously described a rapid and high-resolution separation of a disaccharide mixture of heparin/heparan sulfate (19) and chondroitin sulfate/dermatan sulfate (20) and the disaccharide compositional analysis of these glycosaminoglycans using normal polarity CE. Larger oligosaccharides from heparin and heparan sulfate have also been analyzed using positive or normal polarity CE (21). This paper reports a single method capable of even higher resolution, affording the complete separation of eight heparin/heparan sulfate and eight chondroitin/dermatan sulfate disaccharides. In addition, larger heparin-derived oligosaccharides, ranging in size from disaccharide to tetradecasaccharide, were examined using both normal and reverse polarity CE.

MATERIALS AND METHODS

Materials

The heparin disaccharides (**1h–8h**) having the structure ΔUA2X(1 → 4)-D-GlcNpY6X (where X = H or SO_3^- and Y = SO_3^- or Ac) were obtained from Sigma Chemical (St. Louis, MO) and Grampian Enzymes (Aberdeen, Scotland). The larger heparin-derived oligosaccharides (**8h–20h**) were prepared directly from porcine mucosal heparin by partial enzymatic depolymerization using heparin lyase I and purified by preparative low-pressure gel permeation chromatography followed by preparative strong anion-exchange (SAX) HPLC (22,23). Porcine intestinal heparin (sodium salt, 145 USP U/mg) was from Hepar (Franklin, OH). Heparin lyase I (heparinase I, EC 4.2.2.7), heparin lyase II (no EC assigned), and heparin lyase III (EC 4.2.2.8) were prepared from *Flavobacterium heparinum* in our laboratory and purified to homogeneity (16). Disaccharides ΔUA(1 → 4)-D-GlcNS (**4h**), ΔUA(1→4)-D-GlcNpS6S (**6h**), ΔUA2S(1 → 4)-D-GlcNpS (**7h**), and ΔUA2S(1 → 4)-D-GlcNpS6S (**8h**) were also purified and characterized in our laboratory from heparin sulfate (24–26). The chondroitin sulfate/dermatan sulfate disaccharide standards (**1c–8c**) having the structure ΔUA2X(1 → 3)-D-GalNpAc4X6X (where X = H or SO_3^-) were obtained from Seikagaku America, Inc. (Rockville, MD). The purity of unsaturated disaccharides (**1h–8h** and **1c–8c**) was confirmed by SAX HPLC (24), RPIP HPLC (27), and CE (19–21), while their identities were confirmed by ^{1}H NMR spectroscopy performed at 360 or 600 MHz (23–26).

Sodium borate (decahydrate, 99%) was from Fisher Scientific (Fair Lawn, NJ), sodium dodecyl sulfate (99%) was from BDH chemicals (Poole, England), and phosphoric acid (85%) was from Mallinkrodt (Paris, KY). The pH measurements were obtained on a Beckmann Φ 40 pH meter and calibrated using standard buffer solution (pH 7.0 and 10.0) from Fisher Scientific. All reagents used were analytical grade. Before use all of the buffers were filtered through a Millipore filter (0.42 μm) to remove any particulate material that could block the capillary.

Methods

Preparation of disaccharide standards for analysis. The stock solutions of heparin/heparan sulfate disaccharides and chondroitin sulfate/dermatan sulfates disaccharides containing nonsulfated, monosulfated, disulfated, and trisulfated standards were prepared at 2 mg/ml in distilled water. A portion of each stock solution (1 μg each) was mixed separately to prepare a standard disaccharide mixture. These standard mixtures of heparin/heparan sulfate disaccharides and chondroitin sulfate/dermatan sulfate disaccharides were stored frozen at −70°C.

Preparation of larger heparin-derived oligosaccharides. Porcine mucosal heparin was partially depolymerized using heparin lyase I by quenching the reaction mixture with hydrochloric acid at 30% reaction completion. After the heparin lyase I was removed from the reaction mixture (pH 3.5) with SP-Sephadex, the heparin oligosaccharides were neutralized and the oligosaccharide products (M_r < 3000) were recovered by pressure filtration through a controlled pore membrane (MWCO 3000). The oligosaccharide fraction was loaded onto a Sephadex G 50 (superfine) column (4.8 cm × 1 m) and eluted with 200 mM sodium chloride at 2 ml/min. The fractions containing disaccharide through tetradecasaccharide were collected, evaporated to dryness, desalted

on a Bio-Gel P-2 (50 cm × 0.5 m), eluted with water, and dried by lyophilization. Each fraction was further purified by semipreparative SAX HPLC (25,26), and major peaks were combined, desalted by Bio-Gel P-2 chromatography, and dried by lyophilization. The purity of the heparin-derived oligosaccharides, isolated as white powders, was confirmed by gradient PAGE (28), CE (19–21), and analytical SAX HPLC (22,24–26) and characterized using 1D and 2D NMR spectroscopy (23). The structures of the higher oligosaccharides were further confirmed by depolymerizing with heparin lyases I, II, and III into their disaccharide units (22,23) and analyzing them over CE (19–21). The heparin oligosaccharides isolated using the above procedure are ΔUA2S(1 → 4)-D-GlcNpS6S (**8h**), ΔUA2S(1 → 4)-α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S(1 → 4)-D-GlcNpS6S (**9h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_2$(1 → 4)-D-GlcNpS6S (**10h**), ΔUA2S(1 → 4)-α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S(1 → 4)-α-D-GlcNpS6S(1 → 4)-β-D-GlcAp(1 → 4)-D-GlcNpS6S (**11h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_3$(1 → 4)-D-GlcNpS6S (**12h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_2$-(1 → 4)-α-D-GlcNpS6S(1 → 4)-β-D-GlcAp(1 → 4)-D-GlcNpS6S (**13h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_4$(1 → 4)-D-GlcNpS6S (**14h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_3$(1 → 4)-α-D-GlcNpS6S-(1 → 4)-β-D-GlcAp-(1 → 4)-D-GlcNpS6S (**15h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S(1 → 4)-α-L-IdoAp2S]$_2$(1 → 4)-D-GlcNpS6S-(1 → 4)-α-L-IdoAp-(1 → 4)-α-D-GlcNpS6S (1 → 4)-β-D-GlcAp(1 → 4)-D-GlcNpS6S (**16h**),* ΔUA2S(1 → 4)-[α-D-GlcNpS6S-(1 → 4)-α-L-IdoAp2S]$_5$(1 → 4)-D-GlcNpS6S (**17h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S (1 → 4)-α-L-IdoAp2S]$_4$-α-D-GlcNpS6S-(1 → 4)-β-D-GlcAp(1 → 4)-D-GlcNpS6S (**18h**), ΔUA2S(1 → 4)-[α-D-GlcNpS6S-(1 → 4)-α-L-IdoAp2S]$_6$(1 → 4)-D-GlcNpS6S (**19h**), and ΔUA2S(1 → 4)-[α-D-GlcNpS6S (1 → 4)-α-L-IdoAp2S]$_5$-(1 → 4)-α-D-GlcNpS6S(1 → 4)-β-D-GlcAp-(1 → 4)-D-GlcNpS6S (**20h**). (*The exact positions of the internal glucuronic and unsulfated iduronic acid residues within this oligosaccharide have not been determined.)

The heparin-derived oligosaccharides were accurately weighed and stock solutions (2 mg/ml) of each were prepared in distilled water. A portion (1 μg/1 μl) of these stock solutions was mixed to prepare the standard heparin oligosaccharide mixture. This heparin oligosaccharide mixture was stored frozen at −70°C.

Preparation of Partially Digested Bovine Lung Heparin

A stock solution of bovine lung heparin (20 mg/ml) in distilled water was treated with heparin lyase I (25 mU/mg of substrate) in 5 mM sodium phosphate buffer (500 μl/mg of substrate) at pH 7.1 containing 200 mM sodium chloride for 16 h at 30°C. The depolymerization was terminated at 30% completion (28,29) and the sample was dried and reconstituted in distilled water at a concentration of 2 μg/μl. This depolymerized product mixture containing higher oligosaccharides is the same as that routinely used for mapping experiments on gradient PAGE (33).

Capillary Electrophoresis

The experiments were performed on a Dionex capillary electrophoresis system (Sunnyvale, CA) equipped with a variable wavelength ultraviolet detector. System operation and data handling were fully controlled and chromatograms were integrated using A1-450 chromatography automated software (Version 3.1) from Dionex running on an IBM-compatable personal computer. The CE system was operated in normal polarity by applying the sample at the anode and in reverse polarity by applying the sample at the cathode. In normal polarity the running buffer was 10 mM sodium borate and 50 mM SDS, adjusted to pH 8.80 with 1 N hydrochloric acid. In reverse polarity the separation was carried out using 20 mM phosphoric acid buffer adjusted to pH 3.48 with sodium phosphate buffer. In both modes, the sample was separated and analyzed using a fused-silica (externally coated except where the tube passed through the detector) capillary tube (75 μm i.d, 375 μm o.d, 78 cm long) from Dionex. Before introduction of the sample each capillary was manually rinsed with 0.1 M phosphoric acid (500 μl), 0.5 M sodium hydroxide (500 μl), distilled water (1 ml), and running buffer (500 μl) and inserted into the machine. Samples were loaded with gravity injection, by hydrostatic pressure (45-mm head height) using a 15-s injection period resulting in the injection of 8 nl. Each experiment was conducted at constant voltage (20, 15, 12, or 10 kV). Peaks were identified by cospiking with disaccharide and oligosaccharide standards.

RESULTS AND DISCUSSION

Heparin and heparan sulfate are structurally similar glycosaminoglycans, differing primarily in their relative content of N-acetylglucosamine, O-sulfation, and glucuronic acid (7,9). Exhaustive enzymatic depolymerization of heparin and heparan sulfate with three polysaccharide lyases, heparin lyase I, heparin lyase II, and heparin lyase III, affords eight unsaturated disaccharides containing nonsulfated, monosulfated, disulfated, and trisulfated disaccharides (**1h–8h**). The general structure of these disaccharides is labeled **h** in Fig. 1. These disaccharides have an unsaturated uronic acid residue (ΔUA) at their nonreducing end and thus exhibit an absorbance maxima at 232 nm (ϵ_m 5000–6000

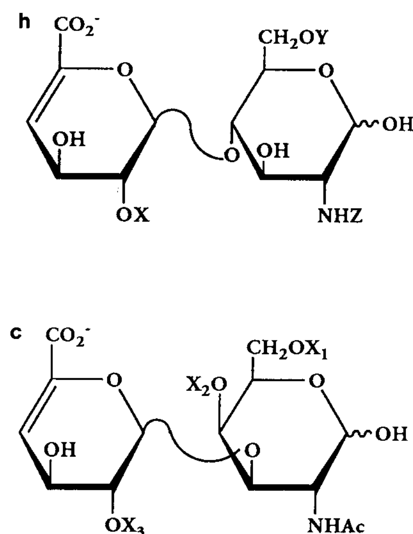

FIG. 1. The disaccharide products formed enzymatically from heparin/heparan sulfate (**1h–8h**) and chondroitin/dermatan sulfate (**1c–8c**) are shown. (h) The heparin/heparan sulfate-derived disaccharides are **1h**, X = Y = H, Z = Ac; **2h**, X = H, Y = SO_3^-, Z = Ac; **3h**, X = SO_3^-, Y = H, Z = Ac; **4h**, X = Y = H, Z = SO_3^-; **5h**, X = Y = SO_3^-, Z = Ac; **6h**, X = H, Y = Z = SO_3^-; **7h**, X = Z = SO_3^-, Y = H; **8h**, X = Y = Z = SO_3^-. (c) The chondroitin sulfate/dermatan sulfate-derived disaccharides are **1c**, $X_1 = X_2 = X_3$ = H; **2c**, X_1 = SO_3^-, $X_2 = X_3$ = H; **3c**, $X_1 = X_3$ = H, X_2 = SO_3^-; **4c**, $X_1 = X_2$ = H, X_3 = SO_3^-; **5c**, $X_1 = X_2$ = SO_3^-, X_3 = H; **6c**, $X_1 = X_3$ = SO_3^-, X_2 = H; **7c**, X_1 = H, $X_2 = X_3$ = SO_3^-; and **8c**, $X_1 = X_2 = X_3$ = SO_3^-.

M^{-1} cm^{-1}) (19–22,27), permitting their detection using ultraviolet spectroscopy.

Different analytical methods have been used to analyze these disaccharide mixtures, including paper chromatography TLC, electrophoresis, cellulose acetate membrane, and HPLC (30–32). While HPLC has many advantages over these other techniques, such as ease of automation, higher resolution, and availability for quantification, a major disadvantage of HPLC is that it requires microgram amounts of sample and is often incapable of resolving disaccharide isomers. CE has all the advantages over HPLC as well as higher resolution and a requirement for significantly less sample (10 to 20 pg) for analysis.

The normal polarity CE separation of the eight heparin/heparan sulfate disaccharides (**1h–8h**) had been optimized in a previous study (19) and these disaccharides could be resolved using 10 mM sodium borate and 50 mM SDS at pH 8.80, at a constant voltage of 12 kV (Fig. 2a). Even under these optimized conditions, many of the disaccharide components showed broad peaks and tailing. In addition, resolution was particularly poor for the monosulfated disaccharide isomers. The application of voltage gradient organic additives (21) resulted in some improvement of peak shape but only to the detriment of resolution. The same disaccharide mixture (**1h–8h**) was prepared and analyzed in 20 mM phosphoric acid buffer, pH 3.48, using reverse polarity (Fig. 2b). Using this buffer system, reverse polarity results in excellent resolution due to increased sharpness of peaks and lack of tailing. The separations of disaccharide mixtures (**1h–8h**) under both normal and reverse polarities were compared using the same capillary size (internal diameter and length), injection mode (gravity injection), and injection time (15 s). In normal polarity CE, the trisulfated disaccharide ΔUA2S(1 → 4)-α-D-GlcNpS6S (**8h**) is the slowest migrating component, while nonsulfated disaccharide ΔUA(1 → 4)-α-D-GlcNpAc (**1h**) was the fastest migrating component. In reverse polarity CE the trisulfated disaccharide (**8h**) became the fastest migrating component, while the nonsulfated disaccharide (**1h**) was slowest migrating component. The positions of all of the disaccharides with intermediate sulfation were similarly altered when the polarity was reversed. Conditions that affected separation under reverse polarity were also examined. Decreasing the voltage across the capillary (from 12 to 8 kV) increased the separation between the monosulfated and disulfated isomers present in the mixture but it also substantially increased the analysis time (from 15 and 26 min to 28 and 39 min). Decreasing the applied voltage to <5 kV resulted in broad peaks and also markedly increased the analysis time; the nonsulfated disaccharide migrated in 6 min at 20 kV and in 100 min at 5 kV. Increasing the length of the capillary (68 to 78 cm) did not significantly affect either the separation or the migration time of the disaccharide mixture.

Similar experiments were performed on the eight chondroitin sulfate/dermatan sulfate disaccharides (**1c–8c**) prepared by complete enzymatic depolymerization of chondroitin sulfates and dermatan sulfate with chondroitin lyase ABC. A sample containing equal amounts (1 μg/μl) of a nonsulfated, three monosulfated, three disulfated, and a trisulfated disaccharide was first analyzed by normal polarity CE using 10 mM sodium borate and 50 mM SDS buffer of pH 8.80 at various constant voltages (10–20 kV). The results of this separation (Fig. 2c) were similar to those previously reported using similar conditions (20). The same disaccharide mixtures were next analyzed by reverse polarity CE in 20 mM phosphoric acid buffer of pH 3.48 through a range of constant voltages (10–20 kV). An electropherogram at 12 kV (Fig. 2d) using reverse polarity shows the complete resolution of the eight disaccharides present in the mixture as well as good peak shape and symmetry. In contrast the electropherogram for the same disaccharide mixture using normal polarity at 12 kV (Fig. 2c) showed considerably less resolution particularly among the three monosulfated disaccharides (**2c–**

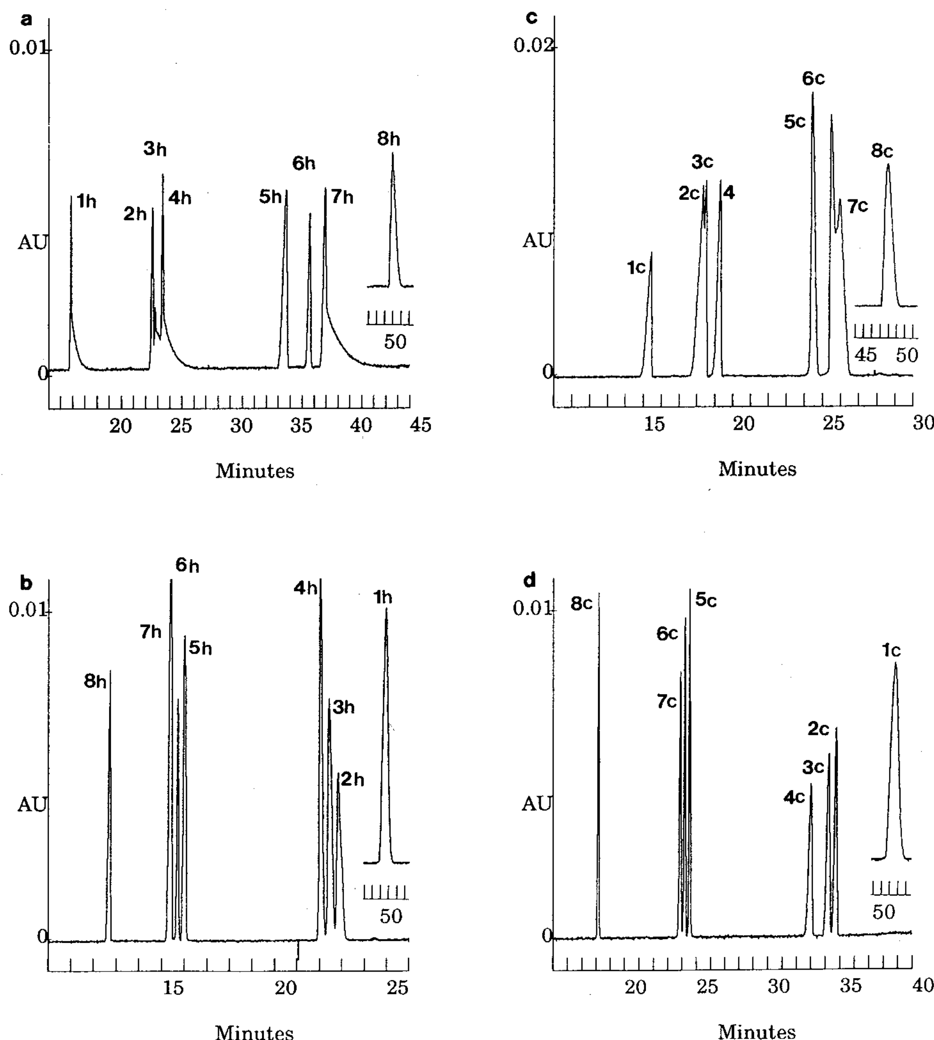

FIG. 2. Capillary electrophoresis of a mixture of nonsulfated, monosulfated, disulfated, and trisulfated disaccharides from heparin/heparan sulfate (a and b) and chondroitin/dermatan sulfate (c and d). (a and c) CE was performed using normal polarity in 10 mM sodium borate and 50 mM SDS, pH 8.80, at 12 kV. (b and d) CE was performed using reversed polarity in 20 mM phosphoric acid buffer, pH 3.48 at 12 kV. In all separations identical capillaries (75 μm i.d. × 375 μm 0.d, 78 cm long) were used and the absorbance full-scale unit was 0.01 at 232 nm. The identity of each disaccharide component in both the heparin/heparan sulfate and chondroitin sulfate/dermatan sulfate disaccharide mixtures, analyzed using normal and reverse polarity CE, was determined by sequentially coinjecting each purified disaccharide component with each disaccharide mixture. The last disaccharide eluting in each electropherogram is shown as an inset in a–d.

4c) and the three disulfated disaccharide isomers (**5c–7c**).

A mixture containing equal amounts of 13 heparin-derived oligosaccharides (1 μg/μl) of sizes ranging from disaccharide through tetradecasaccharide was prepared and subjected to normal polarity CE in 10 mM sodium borate and 50 mM SDS, at pH 8.80 at constant voltages (10–20 kV) (Fig. 3a). These conditions had been previously shown to separate heparin-derived tetrasaccharides and hexasaccharides (23). Normal polarity CE of the mixture of these 13 oligosaccharides resolved nearly all of the components. The peak width of the oligosaccharides was greater than that observed in the disaccharide mixture (Fig. 2a). Varying the potential across the capillary from 5 to 25 kV did not significantly affect the resolution of the oligosaccharides mixture. A reduction in voltage from 20 to 5 kV did prolong the analysis time of each component of oligosaccharide mixture and slightly broadened the peaks. The same oligosaccharide mixture was then analyzed using negative polarity CE in 20 mM phosphoric acid buffer at pH 3.48 using constant voltage (10–20 kV). In contrast to the results obtained on the disaccharide mixtures (Fig. 2), analysis of the oligosaccharide mixture using reverse polarity gave a substantial reduction in resolution (Fig. 3b). Reverse polarity CE was particularly unable to resolve the larger

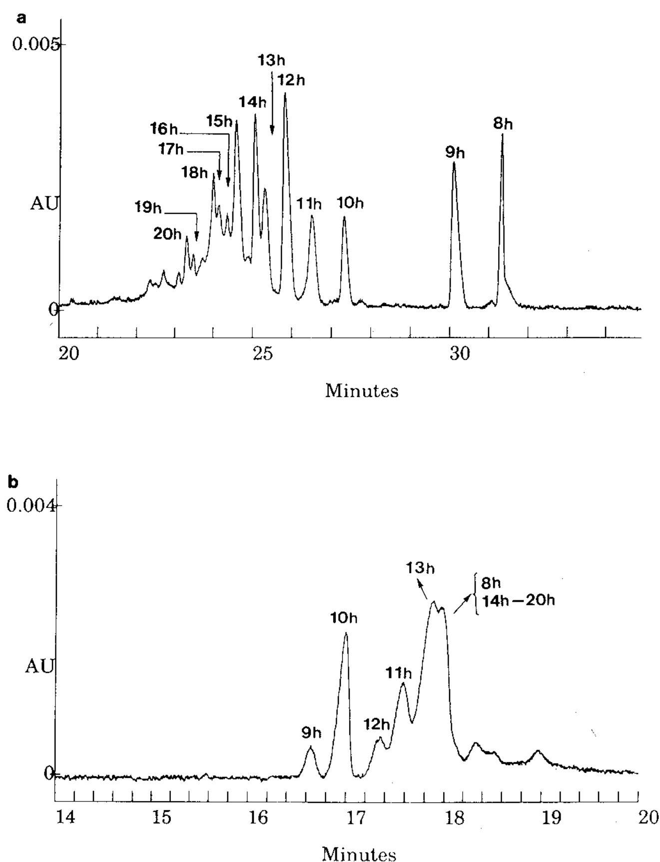

FIG. 3. Oligosaccharide compositional analysis of a mixture of heparin-derived oligosaccharide standards (**8h–18h**). The number of saccharide units in each standard is given in parentheses: **8h** (2); **9h** (4); **10h** and **11h** (6); **12h** and **13h** (8); **14h, 15h,** and **16h** (10); **17h** and **18h** (12); and **19h** and **20h** (14). (a) CE using normal polarity was in 10 mM sodium borate and 50 mM SDS buffer of pH 8.80, at 12 kV. (b) CE using reverse polarity was in 20 mM phosphoric acid buffer, pH 3.48 at 8 kV.

components present in the oligosaccharide mixture (Fig. 3b). Attempts to optimize the reverse polarity CE separation were undertaken. Decreasing the applied voltage from 20 to 3 kV or the pH of buffer from 3.5 to 2.0 did not improve the resolution of the oligosaccharide mixture. Thus, it appears that normal polarity CE provides the best separation of these larger oligosaccharides.

Oligosaccharides obtained from a partial digestion of bovine lung heparin with heparin lyase I were next used to examine the differences between normal and reverse polarity CE. This heparin digest is often used as a standard banding ladder for oligosaccharide analysis on gradient PAGE (28,29,33). This mixture contains oligosaccharide of sizes ranging from disaccharide to oligosaccharides having over 28 sugar units (33). The complexity of this mixture is further increased because of the vastly different molar concentrations of each component present. About 50% of the molar concentration of this mixture corresponds to disaccharide (**8h**) with approximately 25 mol% corresponding to tetrasaccharide and hexasaccharide components. Thus, the larger oligosaccharides (⩾ octasaccharide) correspond to only 25 mol% of the digestion mixture. CE analyses in 10 mM sodium borate and 50 mM SDS at pH 8.80 using normal polarity and 20 mM phosphoric acid buffer at pH 3.48 using reverse polarity are shown in Figs. 4a and 4b. The electropherogram in Fig. 4a shows a separation of the disaccharide through tetradecasaccharide components present in the mixture. This separation is achieved despite the large amount of disaccharide present within the mixture. The separation obtained using reverse polarity CE was significantly poorer, as expected from experiments using the mixture of structurally defined oligosaccharides.

In conclusion, this paper has demonstrated an ex-

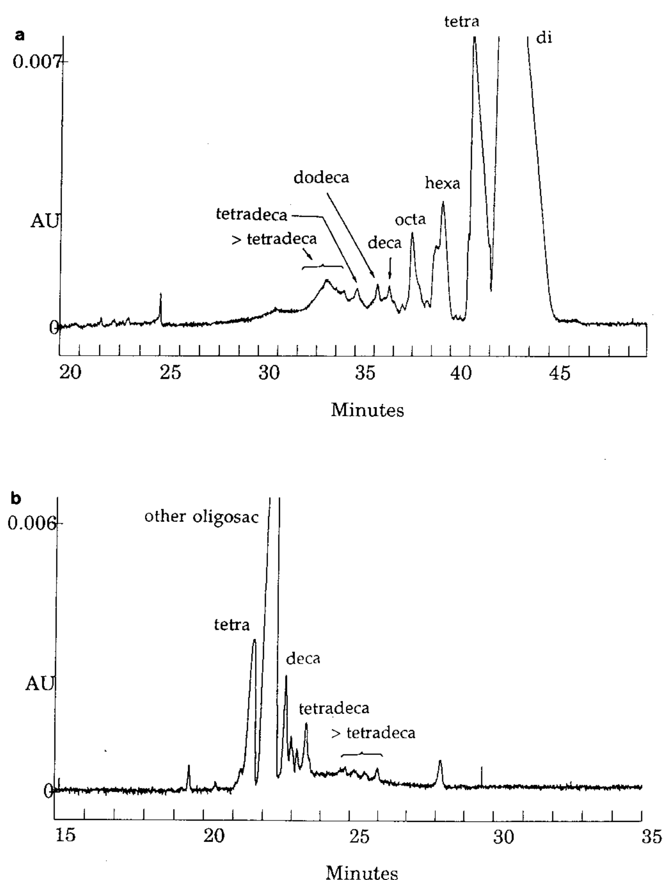

FIG. 4. Capillary electrophoresis of partially heparin lyase I-digested bovine lung heparin. Normal (a) and reverse (b) polarity modes are shown. CE conditions are the same as those described in the legend to Fig. 3. Disaccharide **8h** is the only disaccharide that occurs in significant amounts in this mixture. Undersulfated disaccharides **1h–7h** are obtained in higher concentration from heparan sulfate. The "other oligosaccharide" peak in (b) contains **8h**.

tremely high-resolution separation of mixtures of disaccharides prepared from heparin/heparan sulfate and from chondroitin/dermatan sulfate using reverse polarity CE. This improvement in separation should make the saccharide compositional analysis of glycosaminoglycans much better in terms of both accuracy of identification and sensitivity of detection. Similarly, experiments on larger heparin-derived oligosaccharides showed that it was possible to separate mixtures of oligosaccharides ranging in size and charge from disaccharide (−4) to tetradecasaccharide (−28) using normal polarity CE. Surprisingly, the resolution of reverse polarity separations decreases with increased oligosaccharide size.

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