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Disaccharide Compositional Analysis of Heparin and Heparan Sulfate Using Capillary Zone Electrophoresis

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Capillary zone electrophoresis (CZE) was used to separate eight commercial disaccharide standards of the structure ΔUA2X(1→4)-D-GlcNY6X (where ΔUA is 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid, GlcN is 2-deoxy-2-aminoglucopyranose, S is sulfate, Ac is acetate, X may be S, and Y is S or Ac). These eight disaccharides had been prepared from heparin, heparan sulfate, and derivatized heparins. A similar CZE method was recently reported for the analysis of eight chondroitin and dermatan sulfate disaccharides (A. Al-Hakim and R. J. Linhardt, *Anal. Biochem.* 195, 68–73, 1991). Two of the standard heparin/heparan sulfate disaccharides, having an identical charge of −2, ΔUA2S(1→4)-D-GlcNAc and ΔUA(1→4)-D-GlcNS, were not fully resolved using standard sodium borate/boric acid buffer. This buffer had proven effective in separating chondroitin/dermatan sulfate disaccharides of identical charge. Resolution of these two heparin/heparan sulfate disaccharides could be improved by extending the capillary length, preparing the buffer in ^2H_2O, or eliminating boric acid. Baseline resolution was achieved in sodium dodecyl sulfate in the absence of buffer. The structure and purity of each of the eight new commercial heparin/heparan sulfate disaccharide standards were confirmed using fast-atom-bombardment mass spectrometry and high-field ^{1}H-NMR spectroscopy. Heparin and heparan sulfate were then depolymerized using heparinase (EC 4.2.2.7), heparin lyase II (EC 4.2.2.–), heparinitase (EC 4.2.2.8), and a combination of all three enzymes. CZE analysis of the products formed provided a disaccharide composition of each glycosaminoglycan. As little as 50 fmol of disaccharide could be detected by ultraviolet absorbance.

Heparin and heparan sulfate are highly charged, linear, sulfated polysaccharides that are composed of alternating 1→4-linked glucosamine and uronic acid residues (1). The glucosamine residue in heparin is primarily N-sulfated (D-GlcNS)[2] and the uronic acid is L-iduronic acid–2-sulfate (L-IdoA2S). The most frequently occurring disaccharide sequence in heparin is →4)-α-D-GlcNS6S(1→4)-α-L-IdoA2S(1→. Heparan sulfate contains primarily N-acetylated glucosamine (D-GlcNAc) and glucuronic acid (D-GlcA) and its most common disaccharide sequence is →4)-α-D-GlcNAc(1→4)-β-D-GlcA(1→. Both heparin and heparan sulfate are heterogeneous polymers (1), and thus heparin can contain D-GlcNAc and D-GlcA residues and heparan sulfate often contains substantial D-GlcN and L-IdoA residues. Both show variable O-sulfation at the 3 and 6 positions of D-GlcN and the 2 position of L-IdoA. These glycosaminoglycans (GAGs) exhibit a wide variety of important biological activities (2,3). Heparin, found in mast cell granules, is prepared commercially from beef lung and hog intestine and has been widely used as a clinical anticoagulant for over half a century (4). Heparan sulfate is an important component of extracellular matrix and influences cell growth, development, and differentiation (5,6). Because both heparin and heparan sulfates are microheterogenous, polydisperse mixtures, their structure determination and chemical analysis are difficult. The concentration of heparin, for example, is still most commonly measured by a variety of complicated bioassays (7).

There are three polysaccharide lyases that are capable of acting on heparin and heparan sulfates (8,9). Heparinase (EC 4.2.2.7 or heparin lyase I), heparin lyase II

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[2] Abbreviations used: GAGs, glycosaminoglycans; CZE, capillary zone electrophoresis; L-IdoA, L-idopyranosyluronic acid; D-GlcA, D-glucopyranosyluronic acid; ΔUA, 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid; S, sulfate; GlcN, 2-deoxy-2-aminoglucopyranose; GlcNAc, 2-deoxy-2-acetamidoglycopyranose; FAB, fast atom bombardment; SAX, strong anion exchange; RPIP, reversed-phase ion pairing; PAGE, polyacrylamide gel electrophoresis; TSP, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt; SDS, sodium dodecyl sulfate.

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(no EC number), and heparitinase (EC 4.2.2.8 or heparin lyase III) each act eliminatively to cleave specific linkages (8,10) found within heparin and heparan sulfate and afford a mixture of oligosaccharide products. These oligosaccharide products can be analyzed by strong anion exchange (SAX)-HPLC, (8,11,12) reversed-phase ion pairing (RPIP)-HPLC (13,14), and polyacrylamide gel electrophoresis (PAGE) (8,15,16). The analysis of GAG-derived oligosaccharides prepared using the heparin lyases has been termed oligosaccharide mapping (11,15). A simple and reliable method for determining the disaccharide composition of heparin and heparan sulfate polymers would also be of value both for the study of the chemical structure of these GAGs and for their clinical analysis, particularly if it found applications involving biological fluids and tissues (17).

Recently our group (18) and another (19) described a rapid, high-resolution, and highly sensitive method for determining the disaccharide composition of related but less highly sulfated GAGs: chondroitin sulfates, dermatan sulfate, and hyaluronic acid. These GAGs were first treated with chondroitin lyases and the oligosaccharide products formed were then analyzed by capillary zone electrophoresis (CZE).

We now extend the use of CZE to analyze the disaccharide products obtained on treating heparin and heparan sulfate with individual lyases as well as a mixture of the three polysaccharide lyases. The disaccharide products of these lyases were identified using newly commercially available disaccharide standards.

MATERIALS AND METHODS

Materials

ΔUA(1→4)-D-GlcNS (**4**), ΔUA(1→4)-D-GlcNS6S (**6**), ΔUA2S(1→4)-D-GlcNS (**7**), and ΔUA2S(1→4)-D-GlcNS6S (**8**) were prepared by treating porcine mucosal heparin or heparan sulfate with heparinase, purified by SAX-HPLC, and characterized by FAB-MS and ^{1}H-NMR (8,10,20,21). These four disaccharides together with ΔUA(1→4)-D-GlcNAc (**1**), ΔUA(1→4)-D-GlcNAc6S (**2**), ΔUA2S(1→4)-D-GlcNAc (**3**), and ΔUA2S(1→4)-D-GlcNAc6S (**5**) are newly available commercial standards obtained as a kit from Grampian Enzymes (Aberdeen, Scotland). Using a mixture of heparin lyases, heparin can be used to prepare all eight disaccharides but primarily affords ΔUA(1→4)-D-GlcNS6S (**6**), ΔUA2S(1→4)-D-GlcNS (**7**), and ΔUA2S(1→4)-D-GlcNS6S (**8**); heparan sulfate results primarily in ΔUA(1→4)-D-GlcNAc (**1**), ΔUA(1→4)-kD-GlcNAC6S (**2**), ΔUA2S(1→4)-D-GlcNAc (**3**), and ΔUA(1→4)-D-GlcNS (**4**); fully desulfated, N-resulfated heparin gives primarily disaccharide ΔUA(1→4)-D-GlcNS (**4**); fully desulfated, N-acetylated heparin gives primarily disaccharide ΔUA(1→4)-D-GlcNAc (**1**); and N-desulfated, N-acetylated heparin gives primarily ΔUA2S(1→4)-D-GlcNAc6S (**5**).

Porcine intestinal heparin (sodium salt, 145 USP U/mg) was from Hepar (Franklin, OH). Bovine kidney heparan sulfate (sodium salt) was from Seikagaku American, Inc. (Rockville, MD).

Heparinase (heparin lyase I, EC 4.2.2.7), heparin lyase II (no EC number), and heparitinase (heparin lyase III, or heparan sulfate lyase, EC 4.2.2.8) were prepared fermentatively from *Flavobacterium heparinum* (22) and purified to homogeneity (23). These enzymes are also commercially available from Seikagaku American, Grampian Enzymes, and Sigma Chemical (St. Louis, MO). Oligosaccharide mapping studies performed on heparin and heparan sulfate indicate that the various commercial enzymes as well as those prepared in our laboratory are identical despite differences in their names (8).

Sodium borate was from Fisher Scientific (Fair Lawn, NJ) and boric acid from Mallinckrodt (Paris, KY). ^2H_2O (99.96 at.%), 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt (TSP), and triethanolamine were from Aldrich Chemical (Milwaukee, WI).

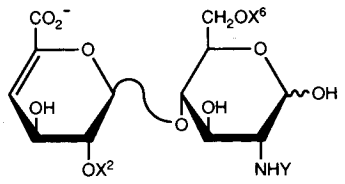
Methods

Characterization of disaccharide standards by ^{1}H-NMR spectroscopy FAB-MS. Each disaccharide was dissolved in ^2H_2O and exchanged three times by freeze drying. The ^{1}H-NMR of each disaccharide (1.5 mg in 0.5 ml) was determined at 360 MHz using a WM360 Bruker NMR spectrometer at 25°C using TSP as an internal standard. Signals were assigned on the basis of reported literature values (10,11,20,21). The purity of each disaccharide was estimated by ^{1}H-NMR on the basis of the presence of signals corresponding to contaminants.

FAB mass spectra were obtained using a VG ZAB-HF double-focusing instrument in the fast-atom-bombardment ionization mode. Disaccharide (15 μg in 1.5 μl of distilled water) was placed on a standard VG stainless steel probe tip containing 2 μl of triethanolamine and the mass spectrum was obtained (24).

Preparation of disaccharide standards for CZE analysis. The disaccharide standards were accurately weighed and a 10 mg/ml stock solution of each was prepared in deionized, distilled water. Portions of these stock solutions were mixed together to prepare standard mixtures and for co-injection studies. The standard solutions were stored frozen at −70°C.

Depolymerization of heparin and heparan sulfate for analysis. Heparin or heparan sulfate (1 mg/0.5 ml) was treated with the appropriate lyase (15 mU of enzyme/mg of substrate) in 5 mM sodium phosphate buffer at pH 7.0 containing 0.2 M sodium chloride for 12 h at 30°C (43°C for heparitinase). Depolymerization was monitored by a change in absorbance at 232 nm.



1. $X^2=X^6=H$, Y=Ac
2. $X^2=H$, $X^6=SO_3^-$, Y=Ac
3. $X^2=SO_3^-$, $X^6=H$, Y=Ac
4. $X^2=X^6=H$, $Y=SO_3^-$,
5. $X^2=X^6=SO_3^-$, Y=Ac
6. $X^2=H$, $X^6=Y=SO_3^-$
7. $X^2=SO_3^-$, $X^6=H$, $Y=SO_3^-$
8. $X^2=X^6=Y=SO_3^-$

FIG. 1. Treatment of heparin or heparan sulfate with an equiunit mixture of heparin lyases I, II, and III leads to the formation of disaccharides of structures **1** through **8.**

Aliquots (10 μl) were removed from the reaction and added to 30 mM hydrochloric acid (1 ml) and the absorbance was measured; the reaction was complete when a constant adsorbance was reached.

CZE analysis. The experiments were performed with a Dionex Capillary Electrophoresis System (Sunnyvale, CA) equipped with a variable-wavelength ultraviolet detector set at 232 nm. Separation and analysis were carried out using a fused silica (externally coated except where the tube passed through the detector) capillary tube (75 μm i.d., 375 μm o.d., 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 and then filled with the operating buffer (i.e., 10 mM sodium borate, 50 mM boric acid, pH 8.8, or 10 mM sodium borate and 50 mM SDS having a pH of 8.5) and inserted into the machine. The sample was injected by gravity injection (15 nl) and electrophoresis was performed at 10, 15, or 20 kV using the operating buffer.

RESULTS AND DISCUSSION

The identity of the commercial disaccharide standards obtained (Fig. 1) was confirmed by FAB-mass spectrometry and their purity assessed using high-field ^{1}H-NMR spectroscopy. The assignments shown in Table 1 and 2 are consistent with the structure of each. Each disaccharide standard was very pure (>90% by NMR). Analysis of each individual disaccharide standard by CZE using 10 mM sodium borate, 50 mM boric acid at pH 8.8, previously described for the analysis of chondroitin and dermatan sulfate disaccharides (18), gave single peaks. All the peaks were symmetrical except for those corresponding to ΔUA2S(1→4)-D-GlcNS (**7**) and ΔUA2S(1→4)-D-GlcNS6S (**8**). These peaks showed substantial tailing, suggesting either the presence of minor contaminants not observed by NMR or a failure to optimize the separation.

An equimolar mixture of the eight disaccharide standards was prepared and analyzed by CZE using 10 mM sodium borate and 50 mM boric acid, pH 8.8, buffer (Fig. 2a). Seven peaks could be observed and were identified by co-injection experiments. Two disaccharides, ΔUA(1→4)GlcNS (**3**) and ΔUA2S(1→4)GlcNAc (**4**), having very similar retention times, comigrated on CZE analysis (Fig. 3a). In an effort to resolve these two components, the pH of the buffer used was adjusted to values between 3.0 and 9.3 and voltages between 10 and 20 kV were tested but this resulted in poorer separations. Lengthening the capillary from 60 to 70 cm or preparing the sodium borate/boric acid buffer in ^2H_2O gave only a modest separation of these two disaccharides (Figs. 3b and 3c). The introduction of 50 mM sodium dodecyl sulfate (19) into 10 mM sodium borate buffer without added boric acid gave optimal resolution of all eight disaccharides (Fig. 2b) but still did not achieve baseline resolution of **3** and **4** (Fig. 3d). Partial resolution of **3** and **4** was accomplished using 6 mM sodium borate and 50 mM SDS at pH 8.5 (Fig. 3e). Baseline resolution of these two disaccharides was achieved in 50 mM SDS in the absence of sodium borate and boric acid (Fig. 3f). Electrophoresis in 50 mM SDS, in the absence of buffer, failed to separate the other six disaccharides. While 50 mM SDS, 10 mM sodium borate at pH 8.8 separated all eight disaccharides (Fig. 2b), this buffer system resulted in a slightly decreased sensitivity. Thus 10 mM sodium borate, 50 mM boric acid at pH 8.8 and 20 kV was selected for the analysis of heparin and heparan sulfate and a second analysis in SDS was used to determine the relative concentrations disaccharides **3** and **4.**

Disaccharide **6,** ΔUA(1→4)-D-GlcNS6S, was prepared at 1 ng/nl and serially diluted up to 1024-fold (each dilution was into an equal volume of deionized, distilled water). The standard curve based, on the first four dilutions shows good linearity (Fig. 4). The peak was detectable (5:1, signal: noise) after a 512-fold dilution corresponding to 29.3 pg or 52 fmol.

Heparin and heparan sulfate (Fig. 1) are structurally similar GAGs differing primarily in their relative content of *N*-acetylglucosamine, O-sulfation, and glucuronic acid (1,25). The analysis of the intact polysaccharides has been largely limited to colorimetric assays (26,27) and bioassays. Other analytical approaches have relied on nitrous acid depolymerization of these GAGs to form oligosaccharide products, which are then reduced with NaB^3H_4 to incorporate radiolabel and analyzed using HPLC.

An alternative approach uses heparinase, an enzyme that acts on either heparin or heparan sulfate through an eliminative mechanism to afford oligosaccharide products with a chromophore that has an absorbance at 232 nm (8,9). Subsequent analysis by HPLC (11,14) or by gradient PAGE (8,15,16) has been used in the study of heparin/heparan sulfate structure as well as their

TABLE 1

H^1-NMR Spectral Assignments for Disaccharide Standards

Compound	ΔUA1[a]	ΔUA2	ΔUA3	ΔUA4	GlcN1[a]	GlcN2	GlcN3	GlcN4	GlcN5	GlcN6,6′	CH_3[b]
1	5.13[c]	3.78	4.22	5.79	5.19	3.87	3.68	3.77	3.84	3.83	2.02
	(5.8)	—[d]	—	(3.5)	(1.6)	—	—	—	—	(3.2)	
2	5.18	3.83	4.30	5.82	5.20	3.86	3.68	3.78	4.14	4.23, 4.34	2.02
	(5.3)	—	—	(3.7)	(3.2)	(7.6)	(7.4)	(6.8)	—	(11.1)	
3	5.14	3.77	4.22	5.79	5.44	3.24	3.73	3.78	3.93	3.8	
	(6.2)	(6.2)	(3.6)	(3.6)	(3.5)	(9.9, 3.5)	(9.9)	(9.1)	(9.1, 3.1)	(3.1)	
4	5.47	4.51	4.29	5.94	5.19	3.84	3.67	3.78	3.94	3.82	2.01
	(2.6)	(2.6)	—	(3.6)	(2.1)	—	—	(9.7)	—	—	
5	5.49	4.54	4.28	5.93	5.19	3.87	3.69	3.78	4.15	4.18, 4.34	2.01
	(3.2)	(3.2)	—	(4.4)	(2.1)	—	—	—	—	(10.4)	
6	5.22	3.86	4.27	5.87	5.48	3.30	3.74	3.84	4.16	4.39, 4.24	
	—	—	(2.2)	(3.7)	(3.6)	(10.4, 3.6)	(9.6)	(9.3)	—	(11.1, 3.6)	
7	5.53	4.57	4.30	6.00	5.46	3.27	3.72	3.80	3.97	3.85	
	(2.4)	—	(4.3)	(4.3)	(3.1)	(10.0, 3.1)	(9.9)	(9.4)	—	(3.1)	
8	5.55	4.58	4.31	5.99	5.44	3.28	3.74	3.86	4.14	4.35, 4.21	
	(3.0)	(3.0)	(4.4)	—	—	(10.3)	(9.1)	(9.6)	—	—	

[a] The number corresponds to the carbon to which the proton is attached.
[b] CH_3 corresponds to the acetamide group found only on disaccarides **1, 2, 4** and **5**.
[c] Chemical shift is presented as ppm determined using TSP as internal standard, and assignments were made using model compounds. The numbers in parentheses are the coupling constants reported in hertz.
[d] Coupling constants could not be determined due to multiplicity or overlap.

analysis. These studies have primarily relied on a single lyase to depolymerize heparin or heparan sulfate to a mixture of disaccharide and higher oligosaccharide products. Although such a mixture of oligosaccharides are easily analyzed by either SAX-HPLC or RPIP-HPLC, the presence of higher oligosaccharides (primarily tetrasaccharides and hexasaccharides) poses a problem for CZE-based analysis. First, although CZE analysis of chondroitin sulfate and dermatan sulfate (18,19) successfully separated disaccharides with up to three sulfate groups (net charge ≥ -4), higher oligosaccharides (net charge > -4), such as those formed from heparin treated with heparinase, could not be separated. Oligosaccharides of net charge > -4 all had migration times nearly identical to a trisulfated disaccharide (net charge $= -4$) under all running conditions examined. Second, no standards are commercially available for heparin- and heparan sulfate-derived tetrasaccharides and hexasaccharides. Because of the different and overlapping specificities of the three lyases that act on heparin and heparan sulfate (8,10) a combination of these three enzymes was examined to reduce the oligo-

TABLE 2

FAB-Mass Spectral Analysis of Disaccharide Standards

Compound	Molecular ions[a] $[M-Na_x + H_{x-1}]$ 0	1	2	3	Fragment ions
1		378			—
2	502	480	458		378 $[M-Na-NaSO_3 + H]^-$
3		480	458		—
4		438	416		—
5		582	560	538	458 $[M-2Na-NaSO_3 + 2H]^-$
6	562	540	518		438 $[M-Na-NaSO_3 + H]^-$
7		540	518		416 $[M-2Na-NaSO_3 + 2H]^-$
8	664	642	620		540 $[M-Na-NaSO_3 + H]^-$, 360 $[M-Na-\Delta UA2S + H]^-$

[a] The molecular ions (M) are listed as the fully sodiated form of the structures shown in Fig. 1.

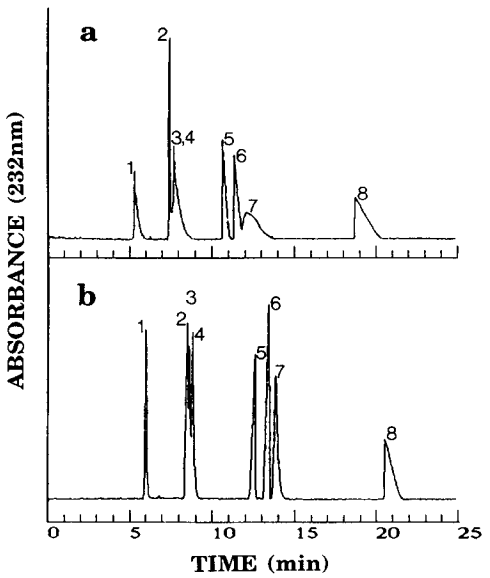


FIG. 2. Electropherograms showing the analysis of a 15-nl mixture containing 1 ng/nl each of disaccharides **1–8.** (a) Analysis was performed using 10 mM sodium borate, 50 mM boric acid, pH 8.8, at 20 kV. (b) Analysis was performed using 50 mM SDS, 10 mM sodium borate, pH 8.5, at 20 kV. The identity of the individual peaks were confirmed by coinjection with standards (not shown).

saccharide products to a simple mixture of disaccharides. These could then be identified using commercially available disaccharide standards.

Heparin was treated with each lyase separately as well as a mixture of the three lyases (Figs. 5a–5d). Heparin is sensitive toward heparin lyase I (heparinase) and gives a broad, major peak with a retention time corresponding to that of trisulfated disaccharide **8** (Fig. 5a). Higher oligosaccharides, having high sulfation, observed on SAX-HPLC analysis (11), comigrate with trisulfated disaccharide **8** on CZE analysis, resulting in increased peak width and peak intensity. The peaks eluting between 13 and 16 min do not correspond to any of the disaccharide standards and may be tetrasaccharides with low sulfation. Heparin is also very sensitive toward heparin lyase II, affording five identifiable disaccharide products (Fig. 5b). SAX-HPLC shows a similar number of disaccharide products (8). As expected from previous studies using SAX-HPLC (8), no oligosaccharide products are observed when heparin is treated with heparin lyase III (heparitinase) (Fig. 5c). Treatment of heparin with an equiunit mixture of the three lyases affords disaccharide products corresponding to all the disaccharide standards except ΔUA(1→4)GlcNAc (**1**). A single, minor (<2.9 mol% of the product mixture), unassignable peak observed at 13.4 min probably corresponds to a lyase-resistant, undersulfated tetrasaccharide (Fig. 5d).

Heparan sulfate was also treated with each lyase as well as the lyase mixture (Figs. 5e–5h). Heparan sulfate is only slightly sensitive to heparinase, resulting in a small amount of trisulfated disaccharide (**8**). Heparin lyase II cleaves heparan sulfate primarily into disaccharides (Fig. 5f) having higher levels of sulfation (two to three sulfates) while heparitinase treatment affords primarily nonsulfated and monosulfated disaccharides (Fig. 5g). The minor unidentified peak at 8.7 min observed in Fig. 5g might correspond to a heparin lyase III-resistant tetrasaccharide. When heparan sulfate is treated with an equiunit mixture of the three lyases (Fig. 5h) each peak is identified and all the disaccharides are observed except ΔUA2S(1→4)-D-GlcNAc6S (**5**).

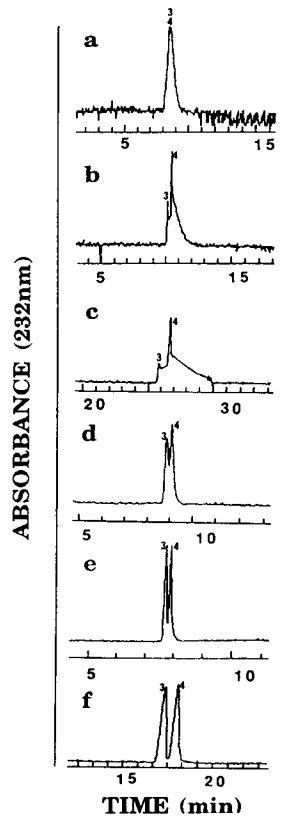


FIG. 3. Electropherograms showing the analysis of a 15-nl mixture containing 1 ng/nl of disaccharides **3** and **4.** (a) The mixture was unresolved using 10 mM sodium borate, 50 mM boric acid buffer, pH 8.8, at 20 kV using a 60-cm capillary. (b) The mixture was partially resolved under conditions identical to those in (a) except using buffer prepared with ^2H_2O. (c) The mixture was partially resolved under conditions identical to those in (b) except using 10 kV. (d) The mixture was partially resolved using 50 mM SDS, 10 mM sodium borate, pH 8.5, at 20 kV. (e) The mixture was better resolved using 6 mM sodium borate containing 50 mM SDS, adjusted to pH 8.6 with sodium hydroxide at 20 kV. (f) Baseline resolution was achieved using 50 mM SDS (in the absence of borate) adjusted to pH 8.6 with sodium hydroxide at 15 kV.

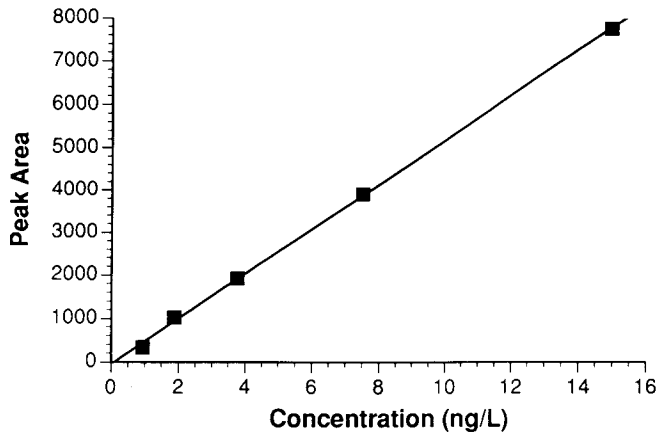


FIG. 4. Standard curve for the analysis of disaccharide **6** showing detector response as a function of amount in nanograms. The slope, *y*-intercept, and *r*-value are 522, −47.9, and 0.999, respectively.

Electropherograms in which heparin and heparan sulfate have been treated with the mixture of lyases (Figs. 5d and 5h) can be used to determine their disaccharide composition. The disaccharide composition of porcine mucosal heparin was (disaccharide, mol% on the basis of relative peak area) ΔUA(1→4)-D-GlcNAc (**1**), 0; ΔUA(1→4)-D-GlcNAc6S (**2**), 2.4; ΔUA2S(1→4)-D-GlcNAc (**3**) and ΔUA(1→4)-D-GlcNS (**4**), 1.9; ΔUA2S(1→4)-D-GlcNAc6S (**5**), 4.8; ΔUA(1→4)-D-GlcNS6S (**6**), 14.4; ΔUA2S(1→4)-D-GlcNS (**7**), 5.2; and ΔUA2S(1→4)-D-GlcNS6S (**8**), 68.3. The disaccharide composition of bovine kidney heparan sulfate was (disaccharide, mol% on the basis of relative peak area) ΔUA(1→4)-D-GlcNAc (**1**), 35; ΔUA(1→4)-D-GlcNAc6S (**2**), 15.7; ΔUA2S(1→4)-D-GlcNAc (**3**) and ΔUA(1→4)-D-GlcNS (**4**), 17.8; ΔUA2S(1→4)-D-GlcNAc6S (**5**), 0; ΔUA(1→4)-D-GlcNS6S (**6**), 11; ΔUA2S(1→4)-D-GlcNS (**7**), 11; and ΔUA2S(1→4)-D-GlcNS6S (**8**), 9.4. Both heparin and heparan sulfate had an equimolar content of disaccharide ΔUA2S(1→4)-D-GlcNAc (**3**) and ΔUA(1→4)-D-GlcNS (**4**). These analyses required 15 ng of polysaccharide and a 20-min analysis time. The results obtained were comparable to the results obtained using SAX-HPLC, requiring 40 μg of sample, specially prepared tetrasaccharide and hexasaccharide stan-

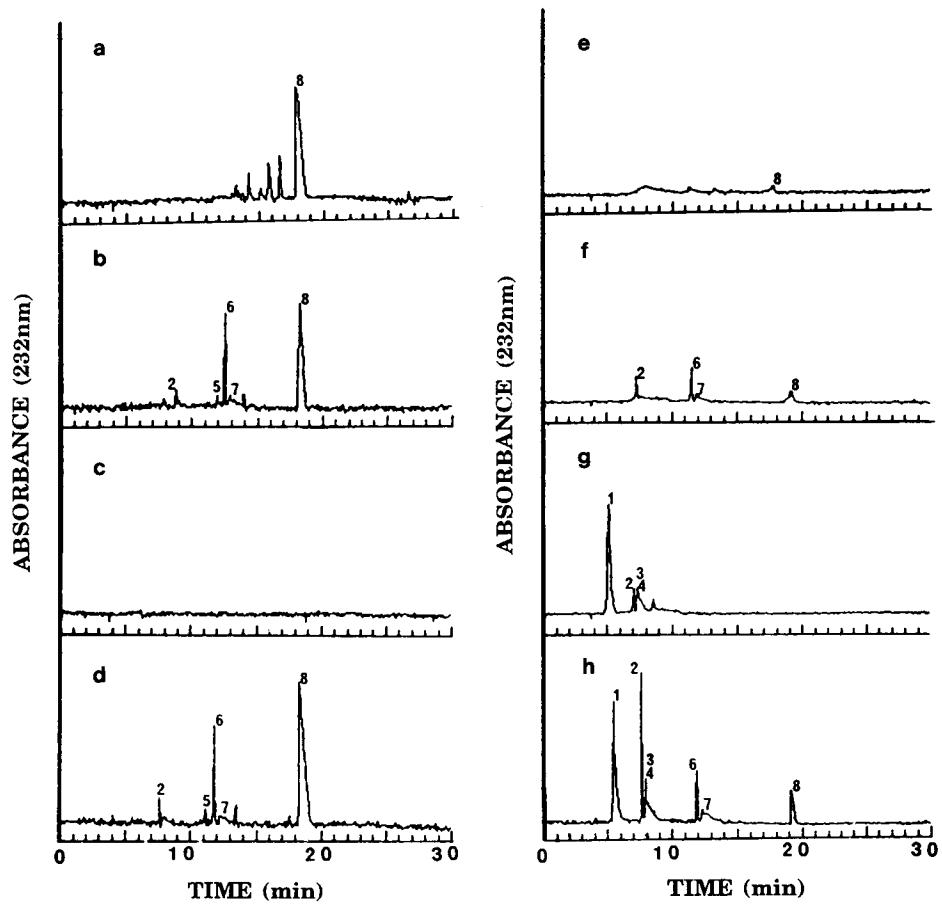


FIG. 5. Electropherograms showing the analysis of lyase-treated heparin and heparan sulfates. Heparin is treated with (a) heparinase, (b) heparin lyase II, (c) heparitinase, and (d) an equiunit mixture of the three lyases. Heparan sulfate is treated with (e) heparinase, (f) heparin lyase II, (g) heparitinase, and (h) an equiunit mixture of the three lyases. Electrophoresis was performed as described under Methods. The identity of individual peaks **1** through **8** was confirmed by coinjection with standards (not shown).

dards, and a 90-min analysis time (8,11). If sufficient sample (~50 ng) is available, full quantitation of heparin- and heparan sulfate-derived disaccharides can best be achieved using two sets of buffer conditions. The first, 50 mM SDS in 10 mM sodium borate, gives baseline resolution of seven of the eight disaccharides (Fig. 2b). The second analysis using 50 mM SDS in the absence of sodium borate results in baseline resolution of the final two components (Fig. 3f).

The application of CZE in combination with commercially available polysaccharide lyase and disaccharide standards represents a valuable new method for the microanalysis of heparin and heparan sulfates. Future studies are aimed at applying these methods to heparin and heparan sulfate isolated from biological samples (including both biological fluids and tissues).

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