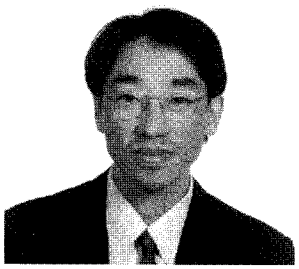
Analytical Biochemistry **283,** 136–145 (2000)
doi:10.1006/abio.2000.4649, available online at http://www.idealibrary.com on IDEAL®

THEMATIC REVIEW

# Electrophoresis for the Analysis of Acidic Oligosaccharides

Mamoru Koketsu and Robert J. Linhardt[1]
*Department of Chemistry, Medicinal and Natural Products Chemistry, Chemical and Biochemical Engineering, University of Iowa, Iowa City, Iowa 52242*



Mamoru Koketsu

Robert J. Linhardt

Carbohydrates are the most abundant species among the biopolymers and have recently been actively studied as important biological molecules (1). Acidic oligosaccharides play especially important biological roles, including control of cell proliferation, differentiation (2, 3), adhesion (4, 5), migration (6), morphological regulation (7, 8) during development (9), blood coagulation (10–12), virus and bacterial infection (13), and tumor malignancy (14). These functions are regulated by the subtle differences in oligosaccharide structure. Heparin, for example, contains a specific pentasaccharide sequence, containing a rare 3-*O*-sulfo glucosamine residue, that is essential for antithrombin III binding, resulting in anticoagulation (15). Understanding oligosaccharide structural microheterogeneity represents an important analytical challenge. Several high-performance liquid chromatography (HPLC) methods, including normal-phase, ion-pairing reversed- phase, and weak anion-exchange chromatography, have been utilized for the separation of glycosaminoglycan-derived oligosaccharides (16–19). However, HPLC generally requires significant volumes of sample and the resolution of structural isomers is not always possible by these chromatographic techniques. Recently, capillary electrophoresis (CE)[2] and polyacrylamide gel electrophoresis (PAGE) have been applied as powerful new tools for the analysis of acidic oligosaccharides. Since electrophoresis is primarily an analytical technique for charged molecules, acidic oligosaccharides that form anions in weakly acidic, neutral, or alkaline media represent ideal analytes. This review focuses on recent developments in using CE and PAGE for the analysis of acidic oligosaccharides.

## ACIDIC CARBOHYDRATES

Naturally occurring acidic oligosaccharides and polysaccharides include carbohydrates that have sulfate esters, sulfamides, phosphate esters, and carboxylate groups. Sialyloligosaccharides, mucins, and glycosaminoglycans including heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronic acid are representative acidic carbohydrates.

Sialic acids are frequently found at the nonreducing terminal of N-linked and O-linked glycoproteins and gangliosides that play a variety of important biological roles (20, 21) (Fig. 1). Mucins are glycoproteins rich in O-linked glycan chains that contain both sialic acid and sulfate esters. Mucins are major glycoprotein components of mucus, covering the luminal surfaces of epithelial respiratory, gastrointestinal, and reproductive tracts. Mucins contain numerous carbohydrate chains attached to the core protein, apomucin, through the linkage GalNAcα1-*O*-Ser/Thr. The mucin glycans determine protein conformation, maintain glycoprotein stability, mediate biological recognition events, and protect sensitive domains from proteolytic attack (22). They are often also required to maintain the physical properties of the glycoproteins, such as biological stability or thermal stability (23, 24). These acidic glycoconjugates also have a major role in protective and barrier functions (25).

Glycosaminoglycans are linear, highly sulfated polysaccharides, typically found O-linked to the core protein of proteoglycans. These glycosaminoglycan chains vary in both length (degree of polymerization of 10–1000) and sulfation, with the exception of keratan sulfate, and consist of a disaccharide repeating unit of uronic acid and amino sugar. There are two major

[1] To whom correspondence should be addressed. Fax: 319-335-6634. E-mail: robert-linhardt@uiowa.edu.

[2] Abbreviations used: CE, capillary electrophoresis; ESI–MS, electrospray ionization–mass spectrometry; MADLI–MS, matrix-assisted laser desorption ionization–mass spectrometry; AGA, 7-amino-1,3-naphthalenedisulfonic acid; PNGase F, peptide *N*-glycosidase F; ANTS, 8-aminonaphthalene-1,3,6-trisufonate.



0003-2697/00 $35.00

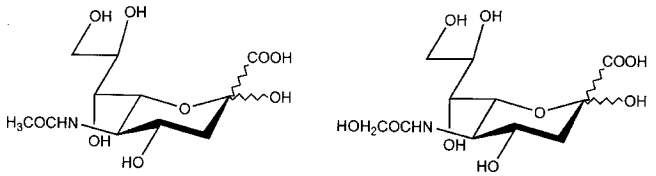



**FIG. 1.** The structures of sialic acids *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc).

classes of glycosaminoglycan: (a) glucosaminoglycans (heparin, heparan sulfate, keratan sulfate, and hyaluronic acid) and (b) galactosaminoglycans (chondroitin sulfate and dermatan sulfate). The uronic acid may be either D-glucuronic acid or L-iduronic acid and the amino sugar may be either N-acetylated or N-sulfated. The amino sugar residues can be substituted with *O*-sulfo groups at C-6 and/or C-3 as can the C-2 of the uronic acid. The incomplete biosynthetic modification of these molecules results in substantial structural complexity (Fig. 2).

## CE OF ACIDIC OLIGOSACCHARIDES

CE has recently emerged as a promising analytical technique that consumes an extremely small amount of sample and that is capable of rapid, high-resolution separation, characterization, and reproducible quantitation of analytes. CE is widely used as a valuable technique for the analysis of proteins, peptides, nucleic acids, and acidic oligosaccharides. The analysis of acidic oligosaccharides is complicated by their extensive isomerism, which results from the geometric diversity of different monosaccharide structures, variable saccharide sequences, different saccharide substituents, and the presence of $\alpha,\beta$ linkages. In the case of oligosaccharides isolated from biological samples, structural analysis must often be carried out with limited amount of analyte. Furthermore, most carbohydrates do not contain natural chromophores and fluorophores. CE is a powerful and highly sensitive analytical tool capable of solving these difficulties.

### *Analysis of Glycosaminoglycan-Derived Disaccharides by UV Detection*

While it is possible to analyze intact glycosaminoglycans using CE (26), they are best analyzed following their controlled enzymatic depolymerization (27). Treatment with polysaccharide lyases affords low-molecular-weight oligosaccharides of reduced structural complexity. These enzymes are eliminases that cleave the glycosidic link between amino sugar and uronic acid residues. The final products of this reaction are disaccharide units containing a $\Delta^4$-uronic acid at the nonreducing terminus. These unsaturated products can be detected by their UV absorbance at 232 nm and are easily separated by CE. CE was first applied to acidic oligosaccharides in the analysis of chondroitin sulfate-, dermatan sulfate-, and hyaluronic acid-derived disaccharides (28–31). Several applications of the analysis of heparin and heparan sulfate disaccharides have being reported. These separations can be performed under either normal or reverse polarity conditions causing analyte to elute with either electroosmotic flow or electrophoresis as the dominant force (32). All known 12 unsaturated disaccharides derived

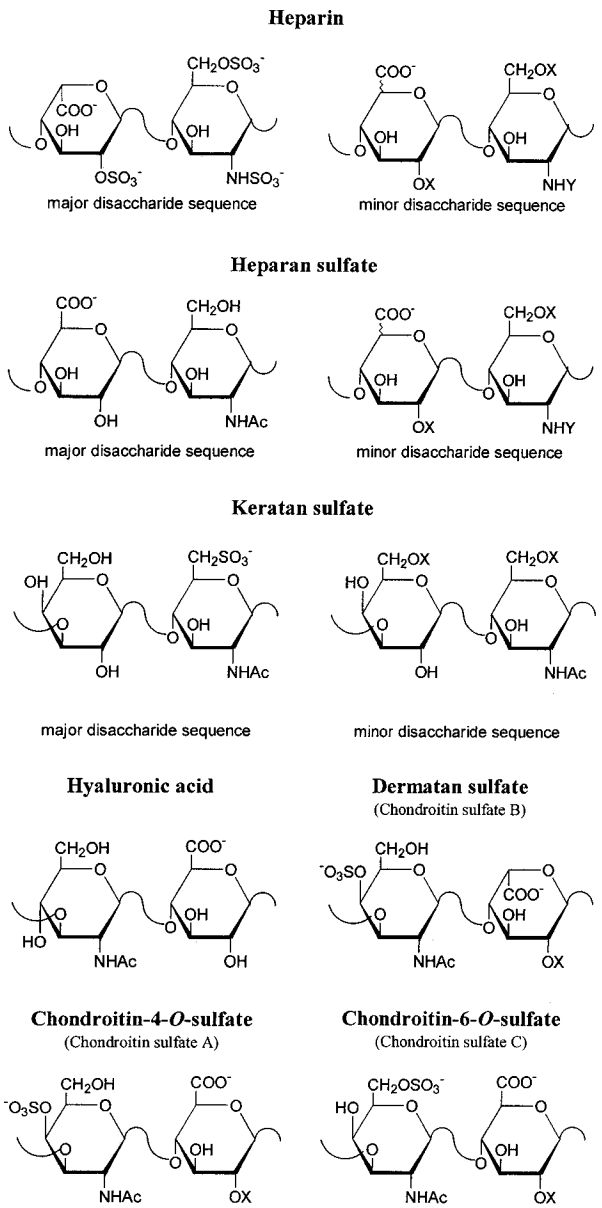
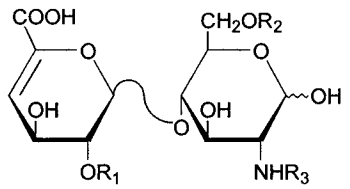



**FIG. 2.** The structures of the major disaccharide repeating unit found in each glycosaminoglycan. Heparin, heparan sulfate, and keratan sulfate have diverse sequences where X is $SO_3^-$ or H and Y is $SO_3^-$, Ac, or H.




| | R1 | R2 | R3 |
|---|---|---|---|
| Nonsulfated | | | |
| 1 | H | H | Ac |
| 2 | H | H | H |
| Monosulfated | | | |
| 3 | H | $SO_3^-$ | Ac |
| 4 | H | $SO_3^-$ | H |
| 5 | $SO_3^-$ | H | Ac |
| 6 | $SO_3^-$ | H | Ac |
| 7 | H | H | $SO_3^-$ |
| Disulfated | | | |
| 8 | $SO_3^-$ | $SO_3^-$ | Ac |
| 9 | $SO_3^-$ | $SO_3^-$ | H |
| 10 | $SO_3^-$ | H | $SO_3^-$ |
| 11 | H | $SO_3^-$ | $SO_3^-$ |
| Trisulfated | | | |
| 12 | $SO_3^-$ | $SO_3^-$ | $SO_3^-$ |

**FIG. 3.** The Δ-disaccharides formed enzymatically from heparin/heparan sulfate (**1–12**).

from heparin and heparan sulfate can be separated into a single run in 5 min at 232 nm (33–35) (Fig. 3) (Fig. 4). Mixtures containing heparin and dermatan sulfate disaccharides can also be separated using fused-silica capillaries through the addition of triethylamine as electrolyte to a running buffer containing borate (36).

The analysis of large oligosaccharides can also be performed by CE. Heparin-derived oligosaccharides that have 2 to 13 saccharide residues are best resolved using normal polarity CE (32). Polysialic acid, oligomers of α-2,8-linked *N*-acetylneuraminic acid (37, 38), of more than 100 saccharide residues and hyaluronic acid oligosaccharides having up to 190 saccharide units are well separated by CE using a combination of a chemically modified capillary and a buffer containing poly(ethylene glycol) with detection at 200 nm (39, 40) (Fig. 5).

### *Labeling Carbohydrates with Chromophores or Fluorophores*

With the exception of unsaturated glycosaminoglycan disaccharides, most carbohydrates have little UV absorbance resulting in very low detection sensitivity. A few carbohydrates such as *N*-acetylglucosamine, *N*-acetylgalactosamine, and sialic acid can be detected in the low UV range at <210 nm. These low-UV wavelengths limit the selection of buffers and additives, and trace impurities can often interfere with such analyses. Sensitive detection methods developed to address this problem include derivatization by fluorescent and/or UV-active chromophores.

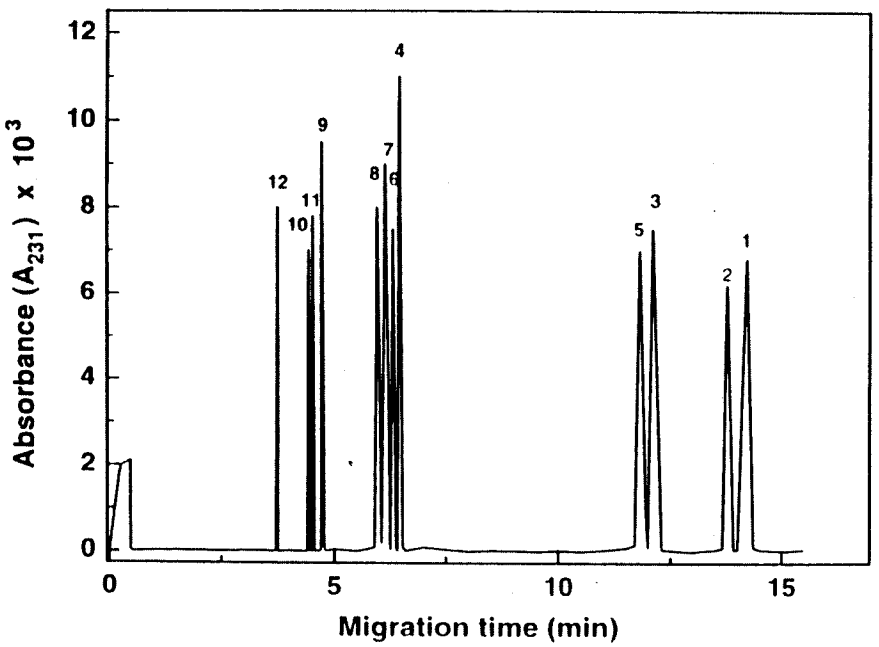



**FIG. 4.** Electropherogram of 12 heparin- and heparan sulfate-derived disaccharides. Peaks corresponded to the structures shown in Fig. 3. Analytical conditions: buffer, 15 mM sodium dihydrogen orthophosphate (pH 3.5); polarity, reversed; applied voltage, 20 kV; temperature, 25°C. Reprinted from N. K. Karamanos *et al.* (*Electrophoresis* **17,** 391–395, 1996) with permission from Wiley–VCH Verlag GmbH.

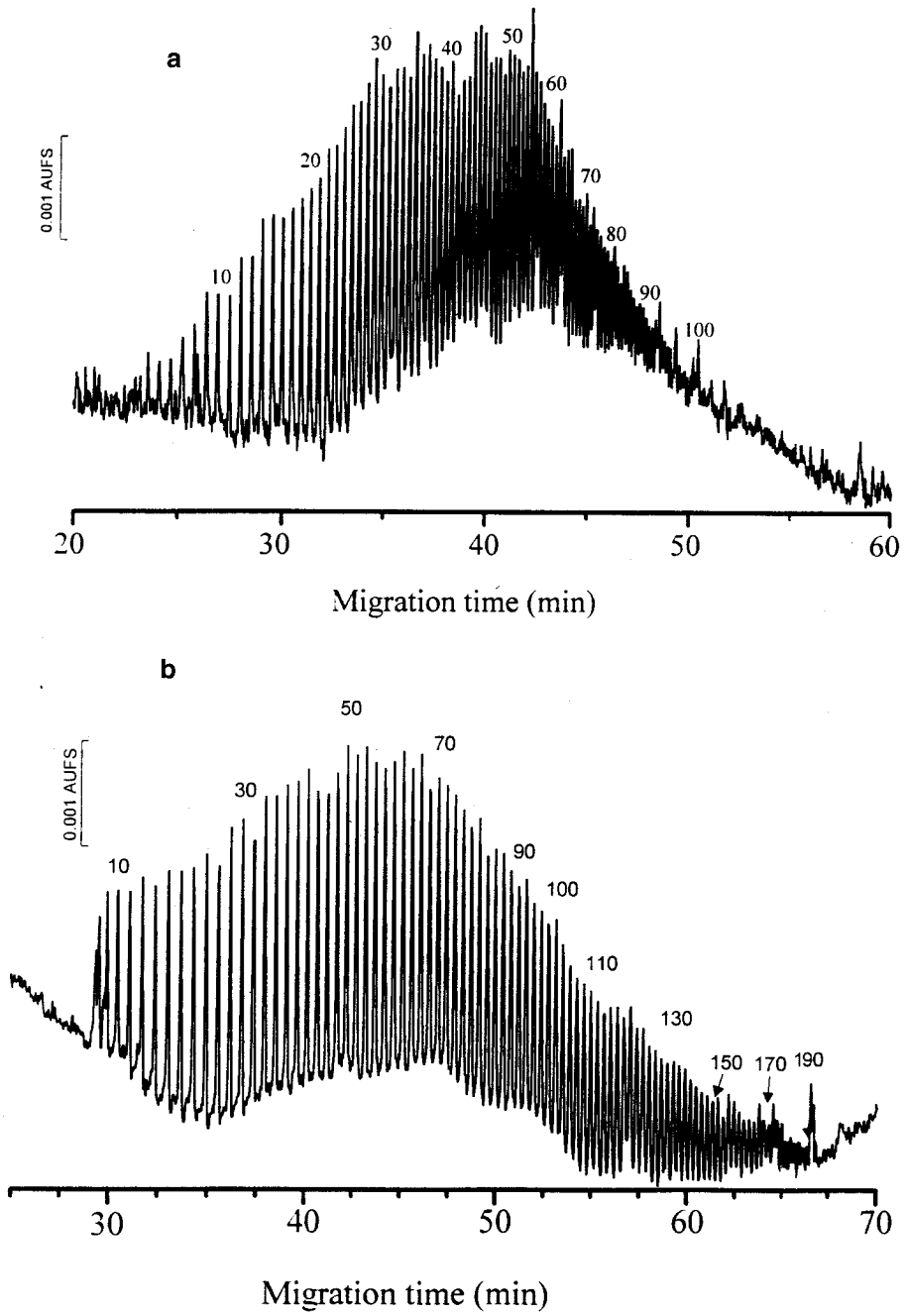



**FIG. 5.** (a) Separation of α-2,8-linked Neu5Ac polymers in the presence of PEG70000 as the neutral polymer. (b) Separation of hyaluronic acid polymers. The numbers indicate the dp of corresponding oligosaccharides. Analytical conditions: capillary, fused-silica coated with 50% phenylmethylpolysiloxane (57 cm × 100 μm i.d.; effective length, 50 cm); buffer, 0.1 M Tris–0.25 M borate (pH 8.5) containing PEG70000 at 10%; applied voltage, 10 kV. Reprinted from K. Kakehi *et al.* (*Anal. Chem.* **71,** 1592–1596, 1999) with permission from the American Chemical Society.

Labeling often results in dramatic changes in the properties of carbohydrates. In addition to providing a useful method for sensitive detection, derivatization of carbohydrates can improve resolution of separation through the introduction of charge and the alteration of analyte. Because most carbohydrates have a reducing end, labeling through a reductive amination reaction between the reducing end of sugar and an amino group of labeling reagent has often been used. Amide bond formation between carboxylate groups present elsewhere in the carbohydrate and the amino group of a labeling reagent, such as carbodiimide, also provides

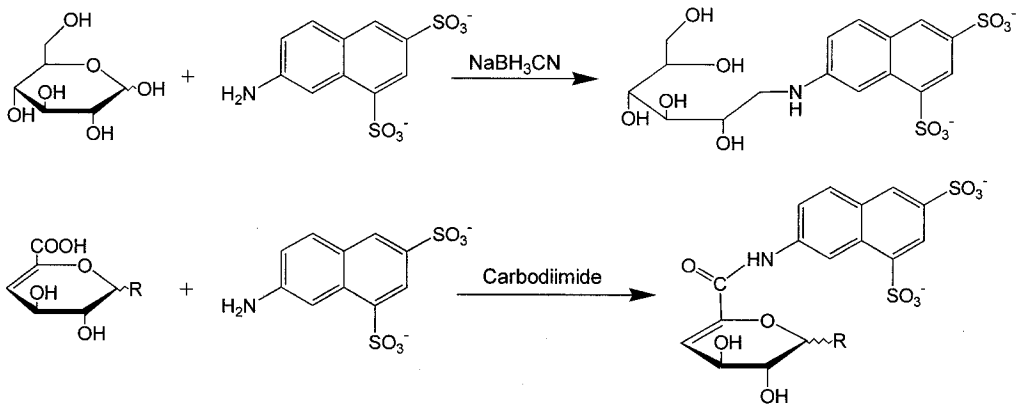



**FIG. 6.** Two standard methods for labeling carbohydrates.

effective detection and separation (41–43) (Fig. 6). Recent reviews (44–47) detail various kinds of labeling reagents and methods for analysis of carbohydrates in CE analysis.

The conversion of neutral oligosaccharides to acidic oligosaccharides through the introduction of fluorescent labels has been intensively investigated for analysis and sequencing (48). Reductive amination of a reducing sugar with 7-amino-1,3-naphthalenedisulfonic acid (AGA) in the presence of sodium cyanoborohydride yields a fluorescent, acidic oligosaccharide derivative that can be efficiently separated by CE. While separation in PAGE is determined by the molecular size of the analytes, the driving force behind the separation by CE is primarily charge based. Thus, CE is often deemed less useful than PAGE for the size determination of the weakly charged carbohydrate (49). This limitation of CE can be circumvented if a dominant charge group is introduced into each oligosaccharide chain. The reductive amination of an uncharged or carboxylated oligosaccharide with a sulfonated fluorescent label provides an oligosaccharide with a net minus charge identical to that of the label at low pH. Under reverse-polarity CE at pH 2.5, only the sulfonate groups (p$I$ < 1) of the fluorescent label remain charged; thus, migration of the labeled oligosaccharide is based on its molecular size. Hyaluronic acid-derived oligosaccharides containing up to 50 saccharide units were separated under these conditions (Fig. 7). A plot of migration time as a function of the log of molecular mass demonstrates a nearly ideal linear relationship (50) (Fig. 8).

New methods for detection, including electrochemical, refractometry, and the use of hyphenated techniques, such as CE–MS (51–53), offer promise in the analysis of acidic oligosaccharides without prior derivatization.

## PAGE ANALYSIS OF ACIDIC OLIGOSACCHARIDES

PAGE analysis of proteins and DNA is one of the most commonly used biochemical techniques. The major limitations for the analysis of carbohydrates using PAGE result from the failure of neutral or weakly

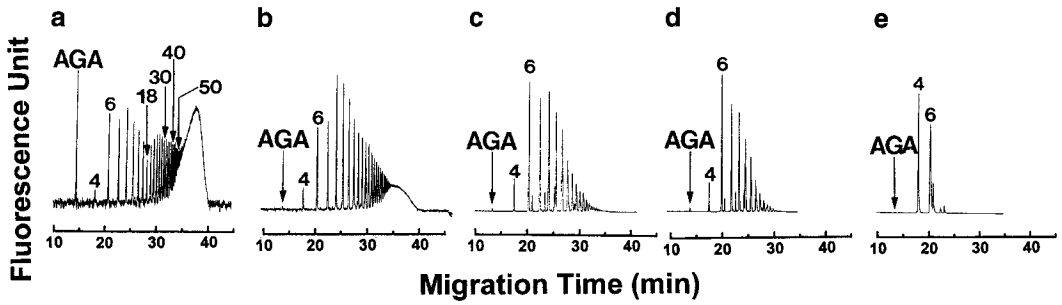



**FIG. 7.** Capillary electropherograms of AGA-labeled oligosaccharide mixtures prepared through controlled depolymerization of hyaluronate with hyaluronate lyase. a, b, c, d, and e correspond to 16.9, 22.5, 43.5, 60.9, and 100% reaction completion, respectively. Each electropherogram contains AGA as an internal standard. The structures of the tetrasaccharide and hexasaccharide in the final product mixture (e) were established by $^1$H NMR analysis. The peaks labeled dp 18, 30, 40, and 50 were assigned by counting from the tetrasaccharide–AGA standard and correspond to peaks of unexpectedly low intensity. Reprinted from Y. Park *et al.* (*Biochim. Biophys. Acta* **1337,** 217–226, 1997) with permission from Elsevier Science.

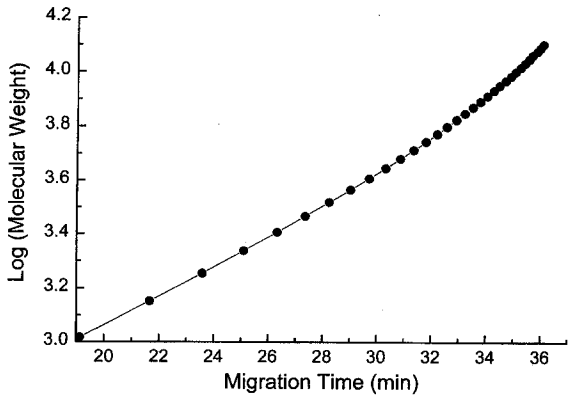



**FIG. 8.** Log molecular weight of oligosaccharide–AGA conjugate as a function of migration time in capillary electrophoresis. These data are taken from Fig. 7a. Reprinted from Y. Park *et al.* (*Biochim. Biophys. Acta* **1337,** 217–226, 1997) with permission from Elsevier Science.

charged molecules to migrate well under electrophoresis and difficulties in the detection of carbohydrates in gels. The solution to these two problems, through the introduction of fluorescent sulfonate-containing labels, has moved PAGE to the forefront as an analytical technique in the carbohydrate field (54). Use of PAGE for analysis of glycosaminoglycans and mixtures of glycosaminoglycan-derived oligosaccharides was also complicated by the requirement for high resolution due to the polydispersity and structural heterogeneity of these samples. Introduction of discontinuous gel electrophoresis together with the development of specialized stacking buffers and gradient gels has solved these problems (55). Gradient PAGE analysis of dermatan sulfate is shown in Fig. 9. Disaccharides that have fewer than three sulfate groups cannot be visualized by silver staining and are more suited to analysis by CE. PAGE analysis is particularly suitable for polysaccharides and high-molecular-weight or highly sulfated oligosaccharides. A combination of Alcian blue and silver staining results in the visualization of all the oligosaccharide products (with the exception of undersulfated disaccharides) that are formed (Fig. 9A). Fluorescent derivatization of the dermatan sulfate glycosaminoglycan released from core protein permits the selective detection of only oligosaccharides that contain the original reducing end (Fig. 9B), facilitating the determination of sequence (Figs. 9C and 9D) (see Ref. 56 for details).

Using the combination of enzymes, including PNGase F and exoglycosidases, with PAGE, ESI–MS, and MALDI–MS analysis, the sequence analysis of N-linked oligosaccharides obtained directly from glycoproteins can be performed. Glycoproteins are first purified by SDS–PAGE and then digested (in-gel) with PNGase F. The released glycans are extracted and analyzed by MALDI–MS (57, 58). SDS–PAGE also is a useful method for process control and quality assessment of recombinant glycoprotein glycosylation (59).

### *Visualization of Nonderivatized Carbohydrates Separated Using PAGE*

Visualization of nonderivatized highly acidic carbohydrate analytes has primarily relied on cationic stains, such as Alcian blue, or the detection of radiolabeled samples by electroelution to positively charged membranes, followed by autoradiography (55, 60–65). Alcian blue detection can be enhanced through silver staining, increasing the detection sensitivity for glycosaminoglycans from microgram to nanogram levels (66–70). Silver staining has a detection limit of 0.04–1 ng for proteoglycans (68).

### *PAGE Analysis of Derivatized Carbohydrates*

Sialylated, phosphorylated, and neutral oligosaccharides that have been labeled with sulfonated fluorophores, such as 8-aminonaphthalene-1,3,6-trisufonate (ANTS), can be separated and analyzed by PAGE. ANTS has three sulfonic acid groups, which give the derivatized sugars a greater net negative charge to improve their migration and resolution. This fluorophore is compatible with SDS and enzymes such as PNGase F, neuraminidase, alkaline phosphatase, and exoglycosidases. ANTS-derivatized sialylated and neutral oligosaccharides are prepared under mild conditions resulting in no oligosaccharide decomposition, often affording a single band on PAGE (71). A recent review details this method of using fluorophore labeling to reduce carbohydrates in PAGE analysis (54). A visible dye, 4-amino-1,1′-azobenzene-3,4′-disulfonic acid, has also been used for PAGE analysis of carbohydrates. Separation of mono-, di-, tri-, and heptasaccharides as well as many monosaccharides, such as fucose, galactose, glucose, mannose, *N*-acetylglucosamine, and *N*-acetylgalactosamine, using gradient PAGE has been achieved with this label (72). Using 2-aminobenzoic acid, labeled heparin or heparan sulfate oligosaccharides could be sequenced rapidly by treating analyte with different combinations of exoenzymes followed by PAGE (73). Two-dimensional gel electrophoresis of fluorescently labeled oligosaccharides is also possible using lectin affinity electrophoresis coupled with PAGE separation (74).

## CONCLUSION

A variety of methods have been developed for the analysis of acidic saccharides by CE and PAGE. These methods allow for both qualitative and quantitative analysis with a high level of sensitivity. Derivatization, commonly used for detection, relies on high-yielding reductive amination. Direct detection is often possible

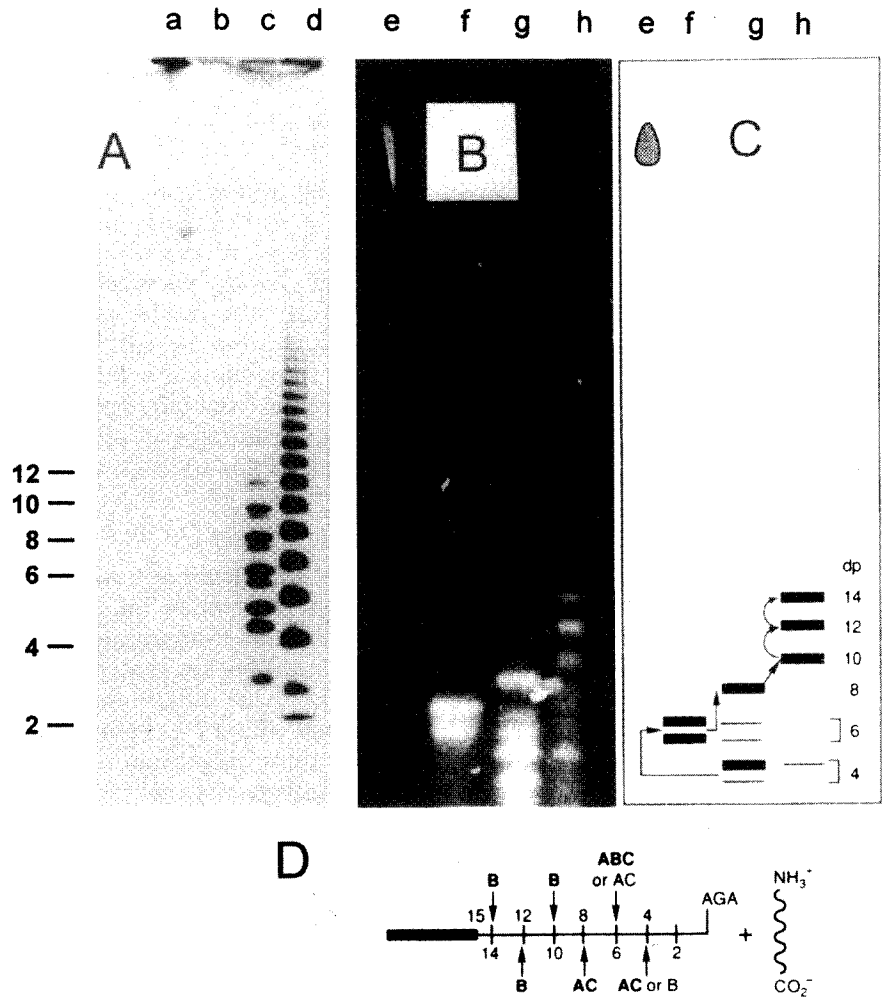



**FIG. 9.** Analysis of dermatan sulfate using gradient PAGE (see Ref. 56 for details). A is a picture of an Alcian blue- and silver-stained PAGE gel of a decorin sample isolated from mature bovine articular cartilage. Lanes: (a) decorin untreated (band at origin) and treated with (b) chondroitin ABC lyase (broken down into monosulfated disaccharides that fail to stain), (c) chondroitin AC lyase, and (d) chondroitin B lyase, respectively (degree of polymerization, given on the left, corresponds to bands of heparin oligosaccharide standards). B is a picture of a transilluminated PAGE gel of dermatan sulfate released from decorin by mild $\beta$-elimination and labeled at the xylose residue with AGA by reductive amination (56). Lanes: (e) AGA-labeled dermatan sulfate untreated and treated with (f) chondroitin ABC, (g) AC, and (h) B lyase, respectively. C shows how the sequence is determined from the banding pattern observed in B. D shows the positioning of linkages susceptible to chondroitin lyases. AGA is attached to the xylose residue corresponding to saccharide unit 1. Unpublished data from our laboratory.

but is usually less sensitive and subject to interference from contaminants frequently accompanying biological samples. New methods for better detection and separation, such as modified capillaries (fused-silica capillaries with hydrophilic coating, octadecyl-sulfonated silica stationary phase, and octadecyl-silica stationary phase) (75–78) and specially prepared gels offer improvements in the analysis of acidic carbohydrates.

## ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants HL52622 and GM38060.

## REFERENCES

1. Varki, A. (1993) Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology* **3,** 97–130.
2. Nelson, R. M., Venot, A., Bevilacqua, M. P., Linhardt, R. J., and Stamenkovic, I. (1995) Carbohydrate–protein interactions in vascular biology. *Annu. Rev. Cell Dev. Biol.* **11,** 601–631.
3. Price, L. K., Choi, H. U., Rosenberg, L., and Stanley, E. R. (1992) The predominant form of secreted colony stimulating factor-1 is a proteoglycan. *J. Biol. Chem.* **267,** 2190–2199.
4. Shur, B. D. (1983) Embryonal carcinoma cell adhesion: The role of surface galactosyltransferase and its 90K lactosaminoglycan substrate. *Dev. Biol.* **99,** 360–372.
5. Wisniewski, H. G., Burgess, W. H., Oppenheim, J. D., and Vilcek, J. (1994) TSG-6, an arthritis-associated hyaluronan binding pro-

tein, forms a stable complex with the serum protein inter-alpha-inhibitor. *Biochemistry* **33,** 7423–7429.

6. Bradbury, M. G., and Parish, C. R. (1989) Receptors on lymphocytes for endogenous splenic glycosaminoglycans. *Immunology* **66,** 546–553.
7. Watanabe, M., Ishikawa, K., and Tatemoto, K. (1998) A culture substratum appropriate for brain cells is a chondroitin sulfate glycosaminoglycan in serum. *Cell Tissue Res.* **291,** 445–454.
8. Yim, S. H., Sherin, J. E., and Szuchet, S. (1993) Oligodendrocyte proteoglycans: Modulation by cell–substratum adhesion. *J. Neurosci. Res.* **34,** 401–413.
9. Nurcombe, V., Ford, M. D., Wildschut, J. A., and Bartlett, P. F. (1993) Developmental regulation of neural response to FGF-1 and FGF-2 by heparan sulfate proteoglycan. *Science* **260,** 103–106.
10. Lindahl, U., Thunberg, L., Backstrom, G., Riesenfeld, J., Nordling, K., and Bjork, I. (1984) Extension and structural variability of the antithrombin-binding sequence in heparin. *J. Biol. Chem.* **259,** 12368–12376.
11. Lindahl, U., Lidholt, K., Spillmann, D., and Kjellen, L. (1994) More to "heparin" than anticoagulation. *Thromb. Res.* **75,** 1–32.
12. Linhardt, R. J., Al-Hakim, A., Liu, J. A., Hoppensteadt, D., Mascellani, G., Bianchini, P., and Fareed, J. (1991) Structural features of dermatan sulfates and their relationship to anticoagulant and antithrombotic activities. *Biochem. Pharmacol.* **42,** 1609–1619.
13. Chen, Y., Maguire, T., Hileman, R. E., Fromm, J. R., Esko, J. D., Linhardt, R. J., and Marks, R. M. (1997) Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nat. Med.* **3,** 866–871.
14. Isogai, Z., Shinomura, T., Yamakawa, N., Takeuchi, J., Tsuji, T., Heinegard, D., and Kimata, K. (1996) 2B1 antigen characteristically expressed on extracellular matrices of human malignant tumors is a large chondroitin sulfate proteoglycan, PG-M/versican. *Cancer Res.* **56,** 3902–3908.
15. Lindahl, U., Backstrom, G., Thunberg, L., and Leder, I. G. (1980) Evidence for a 3-*O*-sulfated D-glucosamine residue in the antithrombin-binding sequence of heparin. *Proc. Natl. Acad. Sci. USA* **77,** 6551–6555.
16. Koshiishi, I., Takenouchi, M., Hasegawa, T., and Imanari, T. (1998) Enzymatic method for the simultaneous determination of hyaluronan and chondroitin sulfates using high-performance liquid chromatography. *Anal. Biochem.* **265,** 49–54.
17. Kim, Y. S., Liu, J., Han, X. J., Pervin, A., and Linhardt, R. J. (1995) Analysis of fluorescently labeled sugars by reversed-phase ion-pairing high-performance liquid chromatography. *J. Chromatogr. Sci.* **33,** 162–167.
18. Toida, T., Huang, Y., Washio, Y., Maruyama, T., Toyoda, H., Imanari, T., and Linhardt, R. J. (1997) Chemical microdetermination of heparin in plasma. *Anal. Biochem.* **251,** 219–226.
19. Volpi, N. (2000) Hyaluronic acid and chondroitin sulfate unsaturated disaccharides analysis by high-performance liquid chromatography and fluorimetric detection with dansylhydrazine. *Anal. Biochem.* **277,** 19–24.
20. Schauer, R., Kelm, S., Roggentin, G., and Shaw, L. (1995) Biochemistry and Role of Sialic Acids, Plenum Press, New York.
21. Schauer, R. (1982) Chemistry, metabolism, and biological functions of sialic acids. *Adv. Carbohydr. Chem. Biochem.* **40,** 131–234.
22. Oh-eda, M., Hasegawa, M., Hattori, K., Kuboniwa, H., Kojima, T., Orita, T., Tomonou, K., Yamazaki, T., and Ochi, N. (1990) *O*-linked sugar chain of human granulocyte colony-stimulating factor protects it against polymerization and denaturation allowing it to retain its biological activity. *J. Biol. Chem.* **265,** 11432–11435.
23. Williamson, G., Belshaw, N. J., Noel, T. R., Ring, S. G., and Williamson, M. P. (1992) *O*-glycosylation and stability. Unfolding of glucoamylase induced by heat and guanidine hydrochloride. *Eur. J. Biochem.* **207,** 661–670.
24. Woodward, H. D., Ringler, N. J., Selvakumar, R., Simet, I. M., Bhavanandan, V. P., and Davidson, E. A. (1987) Deglycosylation studies on tracheal mucin glycoproteins. *Biochemistry* **26,** 5315–5322.
25. Van den Steen, P., Rudd, P. M., Dwek, R. A., and Opdenakker, G. (1998) Concepts and principles of *O*-linked glycosylation. *Crit. Rev. Biochem. Mol. Biol.* **33,** 151–208.
26. Toida, T., and Linhardt, R. J. (1996) Detection of glycosaminoglycans as a copper (II) complex in capillary electrophoresis. *Electrophoresis* **17,** 341–346.
27. Silva, M. E., and Dietrich, C. P. (1975) Structure of heparin. Characterization of the products formed from heparin by the action of a heparinase and a heparitinase from *Flavobacterium heparinum*. *J. Biol. Chem.* **250,** 6841–6846.
28. Carney, S. L., and Osborne, D. J. (1991) The separation of chondroitin sulfate disaccharides and hyaluronan oligosaccharides by capillary zone electrophoresis. *Anal. Biochem.* **195,** 132–140.
29. Ampofo, S. A., Wang, H. M., and Linhardt, R. J. (1991) Disaccharide compositional analysis of heparin and heparan sulfate using capillary zone electrophoresis. *Anal. Biochem.* **199,** 249–255.
30. Al-Hakim, A., and Linhardt, R. J. (1991) Capillary electrophoresis for the analysis of chondroitin sulfate- and dermatan sulfate-derived disaccharides. *Anal. Biochem.* **195,** 68–73.
31. Al-Hakim, A., and Linhardt, R. J. (1991) Electrophoresis and detection of nanogram quantities of exogenous and endogenous glycosaminoglycans in biological fluids. *Appl. Theor. Electrophor.* **1,** 305–312.
32. Pervin, A., Al-Hakim, A., and Linhardt, R. J. (1994) Separation of glycosaminoglycan-derived oligosaccharides by capillary electrophoresis using reverse polarity. *Anal. Biochem.* **221,** 182–188.
33. Karamanos, N. K., Vanky, P., Tzanakakis, G. N., and Hjerpe, A. (1996) High performance capillary electrophoresis method to characterize heparin and heparan sulfate disaccharides. *Electrophoresis* **17,** 391–395.
34. Karamanos, N. K., and Hjerpe, A. (1998) A survey of methodological challenges for glycosaminoglycan/proteoglycan analysis and structural characterization by capillary electrophoresis. *Electrophoresis* **19,** 2561–2571.
35. Lamari, F., Theocharis, A., Hjerpe, A., and Karamanos, N. K. (1999) Ultrasensitive capillary electrophoresis of sulfated disaccharides in chondroitin/dermatan sulfates by laser-induced fluorescence after derivatization with 2-aminoacridone. *J. Chromatogr. B Biomed. Sci. Appl.* **730,** 129–133.
36. Scapol, L., Marchi, E., and Viscomi, G. C. (1996) Capillary electrophoresis of heparin and dermatan sulfate unsaturated disaccharides with triethylamine and acetonitrile as electrolyte additives. *J. Chromatogr. A* **735,** 367–374.
37. Cheng, M. C., Lin, S. L., Wu, S. H., Inoue, S., and Inoue, Y. (1998) High-performance capillary electrophoretic characterization of different types of oligo- and polysialic acid chains. *Anal. Biochem.* **260,** 154–159.
38. Cheng, M. C., Wang, K. T., Inoue, S., Inoue, Y., Khoo, K. H., and Wu, S. H. (1999) Controlled acid hydrolysis of colominic acid under microwave irradiation. *Anal. Biochem.* **267,** 287–293.
39. Kakehi, K., Kinoshita, M., Hayase, S., and Oda, Y. (1999) Capillary electrophoresis of *N*-acetylneuraminic acid polymers and

hyaluronic acid: Correlation between migration order reversal and biological functions. *Anal. Chem.* **71,** 1592–1596.

40. Kakehi, K., Susami, A., Taga, A., Suzuki, S., and Honda, S. (1994) High-performance capillary electrophoresis of *O*-glycosidically linked sialic acid-containing oligosaccharides in glycoproteins as their alditol derivatives with low-wavelength UV monitoring. *J. Chromatogr. A* **680,** 209–215.
41. El Rassi, Z., Postlewait, J., Mechref, Y., and Ostrander, G. K. (1997) Capillary electrophoresis of carboxylated carbohydrates. III. Selective precolumn derivatization of glycosaminoglycan disaccharides with 7-aminonaphthalene-1,3-disulfonic acid fluorescing tag for ultrasensitive laser-induced fluorescence detection. *Anal. Biochem.* **244,** 283–290.
42. Mechref, Y., Ostrander, G. K., and El Rassi, Z. (1995) Capillary electrophoresis of carboxylated carbohydrates. I. Selective precolumn derivatization of gangliosides with UV absorbing and fluorescent tags. *J. Chromatogr. A* **695,** 83–95.
43. Mechref, Y., Ostrander, G. K., and El Rassi, Z. (1995) Capillary electrophoresis of carboxylated carbohydrates. Part 2. Selective precolumn derivatization of sialooligosaccharides derived from gangliosides with 7-aminonaphthalene-1,3-disulfonic acid fluorescing tag. *Electrophoresis* **16,** 1499–1504.
44. Novotny, M. V., and Sudor, J. (1993) High-performance capillary electrophoresis of glycoconjugates. *Electrophoresis* **14,** 373–389.
45. Grimshaw, J. (1997) Analysis of glycosaminoglycans and their oligosaccharide fragments by capillary electrophoresis. *Electrophoresis* **18,** 2408–2414.
46. El Rassi, Z., and Mechref, Y. (1996) Recent advances in capillary electrophoresis of carbohydrates. *Electrophoresis* **17,** 275–301.
47. El Rassi, Z. (1997) Recent developments in capillary electrophoresis of carbohydrate species. *Electrophoresis* **18,** 2400–2407.
48. Lee, K. B., Kim, Y. S., and Linhardt, R. J. (1991) Capillary zone electrophoresis for the quantitation of oligosaccharides formed through the action of chitinase. *Electrophoresis* **12,** 636–640.
49. Carney, S. L., Caterson, B., and Penticost, H. R. (1996) The investigation of glycosaminoglycan mimotope structure using capillary electrophoresis and other complementary electrophoretic techniques. *Electrophoresis* **17,** 384–390.
50. Park, Y., Cho, S., and Linhardt, R. J. (1997) Exploration of the action pattern of Streptomyces hyaluronate lyase using high-resolution capillary electrophoresis. *Biochim. Biophys. Acta* **1337,** 217–226.
51. Figeys, D., and Aebersold, R. (1998) High sensitivity analysis of proteins and peptides by capillary electrophoresis–tandem mass spectrometry: Recent developments in technology and applications. *Electrophoresis* **19,** 885–892.
52. Yang, Q., Benson, L. M., Johnson, K. L., and Naylor, S. (1999) Analysis of lipophilic peptides and therapeutic drugs: On-line-nonaqueous capillary electrophoresis–mass spectrometry. *J. Biochem. Biophys. Methods* **38,** 103–121.
53. He, T., Chandramouli, N., Fu, E., Wu, A., and Wang, Y. K. (1999) Analysis of reduced and oxidized forms of cytochrome c by capillary electrophoresis and capillary electrophoresis–mass spectrometry. *Anal. Biochem.* **271,** 189–192.
54. Jackson, P. (1997) *in* A Laboratory Guide to Glycoconjugate Analysis (Jackson, P., and Gallagher, J. T., Eds.), pp. 113–139, Birkhauser Verlag, Basel, Switzerland.
55. Al-Hakim, A., and Linhardt, R. J. (1990) Isolation and recovery of acidic oligosaccharides from polyacrylamide gels by semi-dry electrotransfer. *Electrophoresis* **11,** 23–28.
56. Liu, J., Desai, U. R., Han, X. J., Toida, T., and Linhardt, R. J. (1995) Strategy for the sequence analysis of heparin. *Glycobiology* **5,** 765–774.
57. Küster, B., Wheeler, S. F., Hunter, A. P., Dwek, R. A., and Harvey, D. J. (1997) Sequencing of *N*-linked oligosaccharides directly from protein gels: In-gel deglycosylation followed by matrix-assisted laser desorption/ionization mass spectrometry and normal-phase high-performance liquid chromatography. *Anal. Biochem.* **250,** 82–101.
58. Küster, B., Hunter, A. P., Wheeler, S. F., Dwek, R. A., and Harvey, D. J. (1998) Structural determination of *N*-linked carbohydrates by matrix-assisted laser desorption/ionization–mass spectrometry following enzymatic release within sodium dodecyl sulphate–polyacrylamide electrophoresis gels: Application to species-specific glycosylation of $\alpha$1-acid glycoprotein. *Electrophoresis* **19,** 1950–1959.
59. Taverna, M., Tran, N. T., Merry, T., Horvath, E., and Ferrier, D. (1998) Electrophoretic methods for process monitoring and the quality assessment of recombinant glycoproteins. *Electrophoresis* **19,** 2572–2594.
60. Desai, U. R., Wang, H. M., and Linhardt, R. J. (1993) Specificity studies on the heparin lyases from *Flavobacterium heparinum*. *Biochemistry* **32,** 8140–8145.
61. Edens, R. E., Al-Hakim, A., Weiler, J. M., Rethwisch, D. G., Fareed, J., and Linhardt, R. J. (1992) Gradient polyacrylamide gel electrophoresis for determination of molecular weights of heparin preparations and low-molecular-weight heparin derivatives. *J. Pharm. Sci.* **81,** 823–827.
62. Rice, K. G., Rottink, M. K., and Linhardt, R. J. (1987) Fractionation of heparin-derived oligosaccharides by gradient polyacrylamide-gel electrophoresis. *Biochem. J.* **244,** 515–522.
63. Turnbull, J. E., and Gallagher, J. T. (1988) Oligosaccharide mapping of heparan sulphate by polyacrylamide-gradient-gel electrophoresis and electrotransfer to nylon membrane. *Biochem. J.* **251,** 597–608.
64. Jandik, K. A., Kruep, D., Cartier, M., and Linhardt, R. J. (1996) Accelerated stability studies of heparin. *J. Pharm. Sci.* **85,** 45–51.
65. Linhardt, R. J., Wang, H. M., Loganathan, D., Lamb, D. J., and Mallis, L. M. (1992) Analysis of glycosaminoglycan-derived oligosaccharides using fast-atom-bombardment mass-spectrometry. *Carbohydr. Res.* **225,** 137–145.
66. Min, H., and Cowman, M. K. (1986) Combined Alcian blue and silver staining of glycosaminoglycans in polyacrylamide gels: Application to electrophoretic analysis of molecular weight distribution. *Anal. Biochem.* **155,** 275–285.
67. Møller, H. J., Heinegard, D., and Poulsen, J. H. (1993) Combined Alcian blue and silver staining of subnanogram quantities of proteoglycans and glycosaminoglycans in sodium dodecyl sulfate–polyacrylamide gels. *Anal. Biochem.* **209,** 169–175.
68. Lyon, M., and Gallagher, J. T. (1990) A general method for the detection and mapping of submicrogram quantities of glycosaminoglycan oligosaccharides on polyacrylamide gels by sequential staining with Azure A and ammoniacal silver. *Anal. Biochem.* **185,** 63–70.
69. Hittner, D. M., and Cowman, M. K. (1987) High-performance gel permeation chromatography of glycosaminoglycans. Column calibration by gel electrophoresis. *J. Chromatogr.* **402,** 149–158.
70. Oreste, P., Peruzzi, M., and Stella, P. (1993) Micromethod for the determination of heparin in plasma after intravenous administration. *Anal. Biochem.* **210,** 136–139.
71. Friedman, Y., and Higgins, E. A. (1995) A method for monitoring the glycosylation of recombinant glycoproteins from conditioned medium, using fluorophore-assisted carbohydrate electrophoresis. *Anal. Biochem.* **228,** 221–225.
72. Westfall, D. A., Flores, R. R., Negrete, G. R., Martinez, A. O., and Haro, L. S. (1998) High-resolution polyacrylamide gel electrophoresis of carbohydrates derivatized with a visible dye. *Anal. Biochem.* **265,** 232–237.

73. Turnbull, J. E., Hopwood, J. J., and Gallagher, J. T. (1999) A strategy for rapid sequencing of heparan sulfate and heparin saccharides. *Proc. Natl. Acad. Sci. USA* **96,** 2698–2703.

74. Linhardt, R. J., Han, X. J., and Fromm, J. R. (1995) Lectin affinity electrophoresis. *Mol. Biotechnol.* **3,** 191–197.

75. Zhang, M., and El Rassi, Z. (1998) Capillary electrochromatography with novel stationary phases. I. Preparation and characterization of octadecylsulfonated silica. *Electrophoresis* **19,** 2068–2072.

76. Zhang, M., and El Rassi, Z. (1999) Capillary electrochromatography with novel stationary phases: II. Studies of the retention behavior of nucleosides and bases on capillaries packed with octadecyl-sulfonated-silica microparticles. *Electrophoresis* **20,** 31–36.

77. Yang, C., and El Rassi, Z. (1998) Capillary electrochromatography of derivatized mono- and oligosaccharides. *Electrophoresis* **19,** 2061–2067.

78. Yang, C., and El Rassi, Z. (1998) Capillary zone electrophoresis of proteins with fused-silica capillaries having polymers and surfactants adsorbed onto surfactant moieties previously covalently bound to the capillary column surface. *Electrophoresis* **19,** 2278–2284.