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Capillary electrophoresis of carboxylated carbohydrates IV.[1] Adjusting the separation selectivity of derivatized carboxylated carbohydrates by controlling the electrolyte ionic strength at subambient temperature and in the absence of electroosmotic flow

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Abstract

The effect of the ionic strength of the running electrolyte on selectivity and resolution of 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) derivatives of carboxylated monosaccharides and sialooligosaccharides derived from gangliosides was evaluated in capillary electrophoresis in the absence of electroosmotic flow and at subambient temperature. The acidic saccharides used in this study were derivatized with ANDSA fluorescing tag to facilitate their detection by laser-induced fluorescence. To maximize resolution among the derivatized saccharides, commercially available fused-silica capillaries with 'zero' electroosmotic flow having polyvinyl alcohol coating on their inner walls were used as the separation capillaries. The effective electrophoretic mobility (μ) of the various ANDSA derivatized mono- and oligosaccharides decreased linearly with the inverse of the square root of the buffer concentration ($1/\sqrt{C}$) used in the running electrolyte. The extent of screening of the charge on the solute by the electrolyte counterions varied among the various saccharides as was manifested by the slopes of the lines of μ vs. $1/\sqrt{C}$. Increasing the ionic strength of the running electrolyte allowed, via its charge screening effect, the modulation of selectivity thus adjusting the resolution of closely related saccharides. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Buffer composition; Carbohydrates; Aminonaphthalenedisulfonic acid; Gangliosides

1. Introduction

The analysis of carbohydrates and glycoconjugates by capillary electrophoresis (CE) has rapidly become a complementary approach to the more traditional liquid phase separation methods such as high-performance liquid chromatography and polyacrylamide gel electrophoresis. In fact, capillary electrophoresis of carbohydrates has grown substantially over the past six years as described in recent books and reviews [1–4]. Major advances have been made including novel detection schemes and electrolyte systems specially tailored for the separation and determination of a wide range of carbohydrates [1–4].

Although noncomplexing electrolyte systems have found some use in the CE of carbohydrates, the bulk of CE separation is still accomplished primarily by borate complexation regardless whether the carbohydrates are derivatized or underivatized [2–4]. This is because borate complexation has been shown, since the early stages of traditional electrophoresis, to magnify small structural differences among closely

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[1] Parts I, II and III are [9,6,7], respectively.

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related saccharides, thus providing the selectivity required for successful separation.

As will be shown in this report, noncomplexing electrolyte systems are also suitable for the separation of closely related carbohydrates. With noncomplexing electrolytes, the CE selectivity can be readily manipulated by the ionic strength of the running electrolyte while maintaining a reduced electroosmotic flow and using a constant subambient temperature. It is also shown that the labelling of carbohydrates with a multiply charged tag such as 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) facilitates the detection of the analytes at low levels by laser-induced fluorescence detection and provides the charges for the rapid separative transport.

2. Materials and methods

2.1. Reagents and materials

Gangliosides G_{M2}, G_{M3} and G_{D3} were purified in our laboratory as previously described [5]. Monosialoganglioside (G_{M1}), disialogangliosides (G_{D1a}, G_{D1b} and G_{D3}) and trisialoganglioside (G_{T1b}) were obtained from Matreya (Pleasant Gap, PA, USA). Ceramide-glycanase was obtained from V-Labs (Covington, LA, USA). The following acidic monosaccharides: L-glyceric acid, D-gluconic acid, D-galacturonic acid, and *N*-acetylneuraminic acid (NeuAc), as well as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) were purchased from Sigma (St. Louis, MO, USA). The derivatizing agent, 7-aminonaphthalene-1,3-disulfonic acid (ANDSA), was purchased from TCI America (Portland, OR, USA). Reagent grade sodium phosphate monobasic, hydrochloric acid and sodium hydroxide were obtained from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Instruments and capillaries

A Beckman P/ACE instrument (Fullerton, CA, USA), Model 5010 equipped with an Omnichrome (Chino, CA, USA) Model 3056-8M He-Cd laser multimode, 8 mW at 325 nm and a laser headcoupler to a standard SMA-906 fiber connector was used. The fluorescence emission band-pass filter of 420±2 nm and the cut-on filter of 400 nm for rejecting the laser beam were purchased from Corion (Holliston, MA, USA). The instrument was equipped with a data-handling system comprising an IBM personal computer and a System Gold software. The resulting signal was fed to the computer for storage and real-time display of the electropherograms. The PVA-I coated capillaries were obtained from Beckman and had the dimensions of 40 cm (to detector)/47 cm (total length)×50 μm I.D.×365 μm O.D. The temperature of the capillary was maintained at 15°C by the instrument thermostating system unless otherwise mentioned. Samples were pressure injected at 0.034 bar (i.e., 3.5 kPa).

2.3. Cleavage of sialooligosaccharides from gangliosides

Sialooligosaccharides (for structures, see Fig. 1) were cleaved from gangliosides according to the procedure used previously [6]. First, mixtures containing 100 μg of the ganglioside, 150 μg sodium cholate, and 0.5 units of ceramide-glycanase (1 unit hydrolyzes 1 nmol of ganglioside per min at pH 5.0, 37°C) in 200 μl of 50 m*M* sodium acetate buffer, pH 5.0, were incubated at 37°C and monitored at 1 h intervals by thin-layer chromatography. The action of the enzyme was terminated after 4 h by the addition of 1.0 ml of chloroform–methanol (2:1, v/v) followed by centrifugation at 7000 *g*. Following centrifugation, the aqueous layer was removed and the procedure repeated twice. The sialooligosaccharides retained in the aqueous layers were combined and dried under nitrogen purge.

The structures of the sialooligosaccharides derived from gangliosides used in this study as well as the charge-to-mass ratios of their ANDSA derivatives are shown in Fig. 1. Two of the sialooligosaccharides, namely sialooligo-G_{D1a} and G_{D1b}, are structural isomers that only differ in the position of the sialic acid residues.

2.4. Derivatization of carboxylated carbohydrates

The seven sialooligosaccharides cleaved from gangliosides (see Fig. 1) and the acidic monosac-

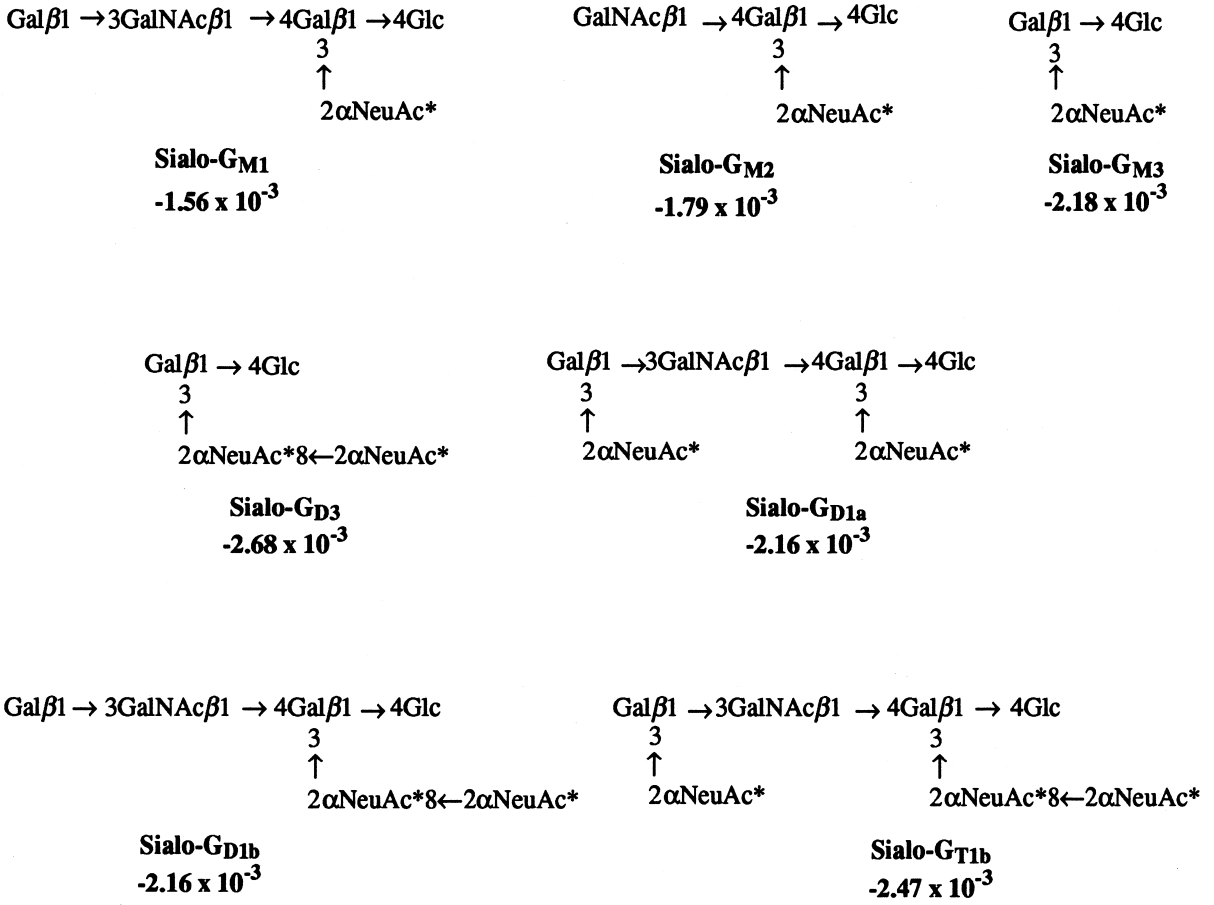

Fig. 1. Structures, abbreviations and charge-to-mass ratios of the ANDSA-sialooligosaccharides derived from gangliosides. The tagging with ANDSA occurs on the carboxylic acid groups of the sialic acid residues (NeuAc) that are indicated by asterisks.

charides (see Fig. 2) were tagged with ANDSA as previously described [6–9]. Briefly, an aliquot of 5 μl of 100 m*M* aqueous solution of EDAC, pH 5.0, was initially added to low μg amounts (<20 μg) of analytes dissolved in distilled water. Then, 10.0 μl of 100 m*M* aqueous solution of the derivatizing agent were added, and the mixture was stirred for 2.0 h at room temperature. Subsequently, the entire reaction mixture containing the derivatized sialooligosaccharides, excess derivatizing agent, and other components of the reaction mixture was analyzed by CE. The excitation and emission spectra of ANDSA as well as of ANDSA derivatized carbohydrates were reported earlier [7].

3. Results and discussion

Throughout this study PVA coated capillaries were used in order to eliminate the electroosmotic flow (EOF) and in turn maximize resolution between closely related saccharides. The migration behavior of the derivatized saccharides in the absence of the EOF was examined with running electrolytes of sodium acetate, pH 5.0, at various ionic strength while maintaining the temperature of the separation capillary at 15°C. The sugars under investigation were labelled with ANDSA via the labelling procedures introduced by our laboratory [6,8–10]. This precolumn derivatization replaces the carboxylic acid

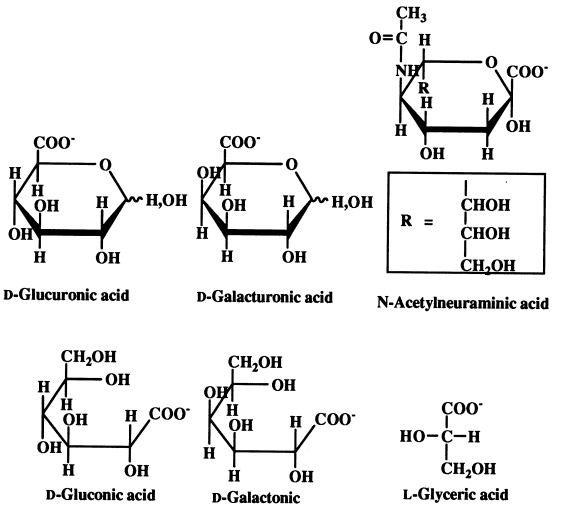

Fig. 2. Structures of the acidic monosaccharides used in this study. The tagging with ANDSA occurs at the carboxylic acid group of the sugars.

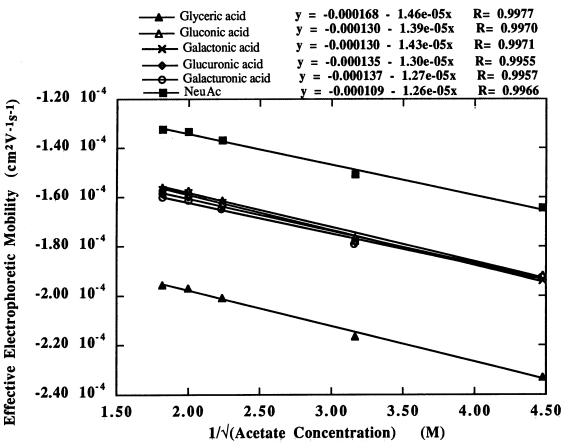

Fig. 3. Plots of the effective electrophoretic mobilities of ANDSA-acidic monosaccharides versus the reciprocal of the square root of sodium acetate concentration in the running electrolyte. Conditions: separation electrolyte, various sodium acetate concentration, pH 5.0; voltage, −20 kV; temperature, 15°C; capillary, PVA-I coated capillary, 40/47 cm×50 μm I.D.×365 μm O.D.

group of the sugars with two sulfonic acid groups which are fully ionized even in very acidic media; therefore, changing the pH of the running electrolyte did not substantially influence the separation of the various analytes.

3.1. Acidic monosaccharides

The effect of the ionic strength of the running electrolyte on the separation of closely related structures was first examined with acidic monosaccharides derivatized with ANDSA, see Fig. 2. These were glyceric acid (oxidized aldotriose), gluconic and galactonic acids (aldonic acids), glucuronic and galacturonic acids (uronic acids) and NeuAc (a sialic acid) derivatized with ANDSA.

Generally, the electrophoretic mobility of a charged particle in an electric field is: (i) directly proportional to its charge, which is dependent on the composition of the separation electrolyte, and (ii) is inversely proportional to the translational friction coefficient, which is a function of the particle shape or size (hydrodynamic volume) and the viscosity of the running electrolyte. While the shape cannot be altered easily, the charge of a solute can be varied readily by the composition of the running electrolyte.

Varying the ionic strength of the running electrolyte affected migration, selectivity and resolution. The results are illustrated in Fig. 3 in terms of effective electrophoretic mobility versus the reciprocal of the square root of the concentration (C) of sodium acetate in the running electrolyte ($1/\sqrt{C}$). As can be seen in Fig. 3, linear plots are obtained with R values greater than 0.995. This corroborates earlier findings by Issaq et al. [11] who formulated an expression that showed the linear dependence of the effective electrophoretic mobility of solutes on $1/\sqrt{C}$. As expected, the electrophoretic mobilities decreased as the ionic strength of the separation electrolyte increased. In the presence of an electrolyte, the effective charge of the solute is less than its actual charge due to the screening effect of the electrolyte counterions. As the screening effect increases with increasing the ionic strength, the solute effective charge will decrease and in turn its electrophoretic mobility will decrease too. The screening effect take places within a stagnant layer immediately adjacent to the surface of the solute. This stagnant layer, which has a thickness on the order of a few molecular radius, is caused by the viscous force acting between the solvent and the surface of the solute [12].

As can be seen in Fig. 4, at 50 mM acetate concentration in the running electrolyte, the aldonic

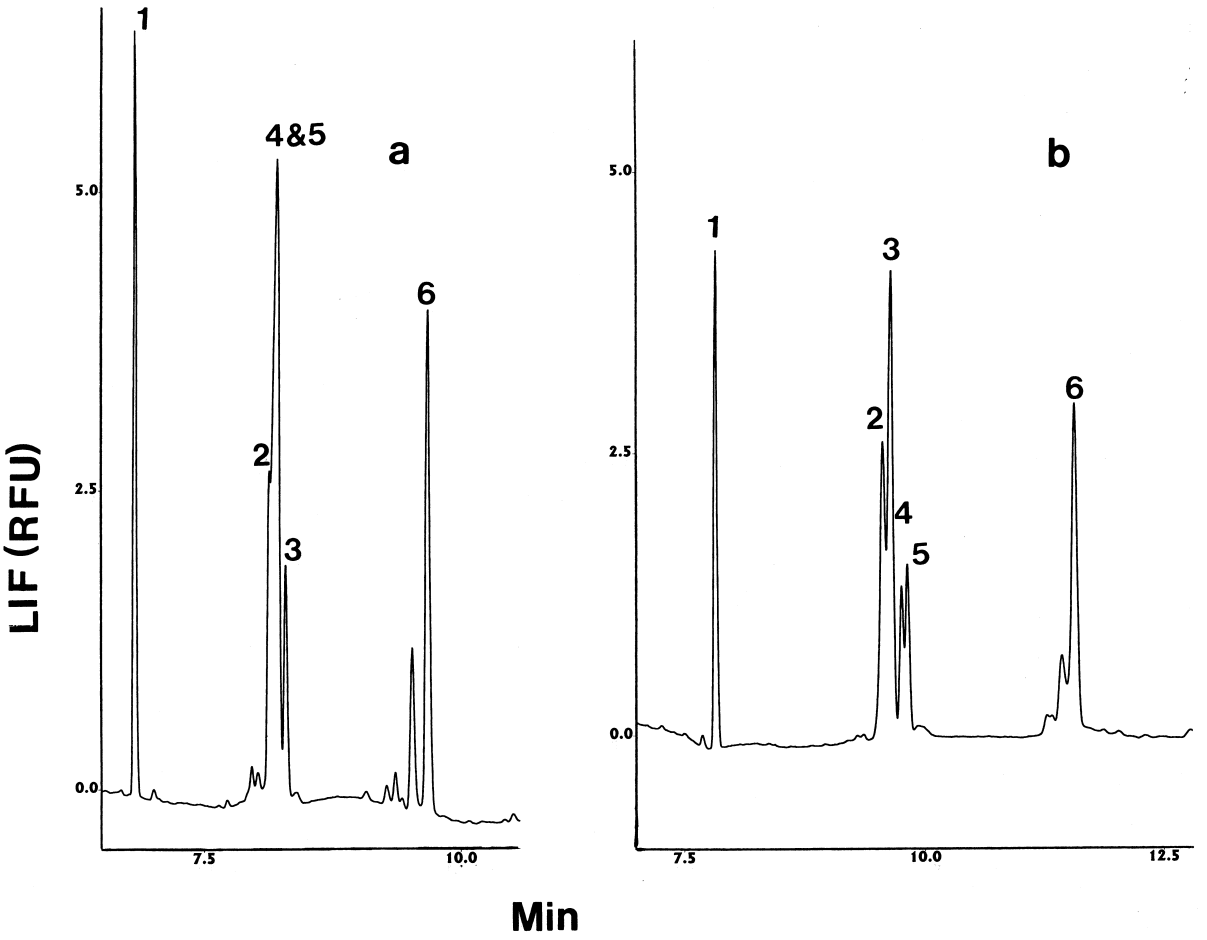

Fig. 4. Electropherograms of ANDSA-acidic monosaccharides at various ionic strengths of the running electrolyte. Conditions: (a) running electrolyte, 50 m*M* sodium acetate, and (b) 300 m*M* sodium acetate, pH 5.0. Other conditions as in Fig. 3. Peak assignments: 1, ANDSA-glyceric; 2, ANDSA-gluconic; 3, ANDSA-galactonic; 4, ANDSA-glucuronic; 5, ANDSA-galacturonic; 6, ANDSA-NeuAc. The peak of excess ANDSA (not shown) appeared at 5.30 and 5.80 min in (a) and (b), respectively.

(i.e., gluconic and galactonic) resolved form each other while the two uronic acids almost coeluted. At acetate concentration of 100 m*M* and above, the uronic acids under investigation, which are two mass units lighter than the aldonic acids showed slower electrophoretic mobility, thus exhibiting different selectivity and migration order than at low acetate concentration. This may be due in part to the fact that the aldonic acids are linear structures while the uronic acids assume the pyranose and the linear forms (see Fig. 2).

A peculiar behavior is the fact that increasing the ionic strength had an opposite effect on the selectivity ($\Delta\mu/\mu_{av}$) and the resolution of the two aldonic acids when compared to the two uronic acids utilized in this study (see Fig. 5). Increasing the ionic strength increased the resolution and selectivity of the uronic acids, while it decreased the resolution and selectivity of the aldonic acids. This may be attributed to differences in conformation (linear vs. cyclic) as well as in the stereochemistry of the hydroxyl groups. This is a confirmation of the fact that the counterion shielding effect depends on the nature of the solute. In addition, and as can be seen in Fig. 3, the screening effect is more pronounced in the case of the smallest solute (i.e., ANDSA-glyceric

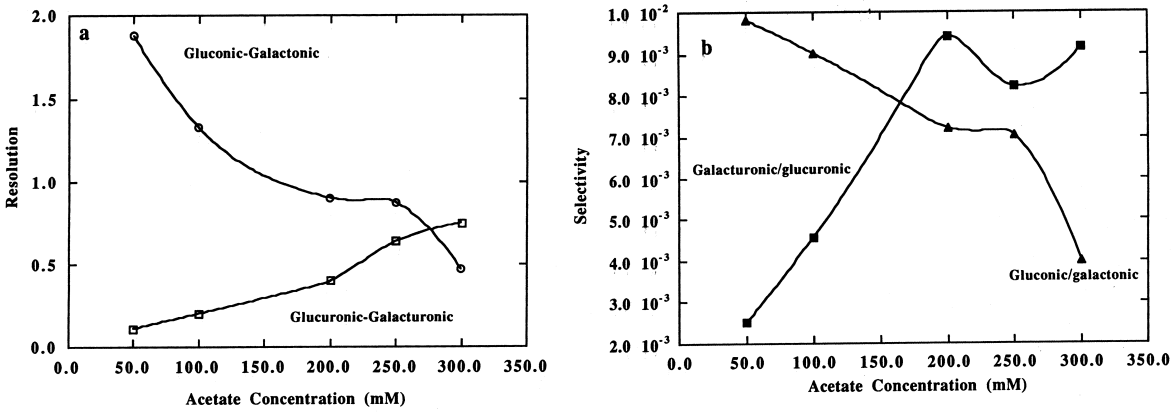

Fig. 5. Plots of (a) the resolution, and (b) selectivity of ANDSA derivatized D-gluconic/D-galactonic acids and D-glucuronic/D-galacturonic acids as a function of the sodium acetate concentration in the running electrolyte. Conditions as in Fig. 3.

acid) than the largest solute (i.e., ANDSA-NeuAc). This is manifested by the more negative slope for ANDSA-glyceric acid as compared to that of ANDSA-NeuAc (−1.46 vs. −1.25). Also, the screening effect is higher for cyclic than linear monosaccharides. This is readily seen by comparing the more negative slopes for galacturonic and glucuronic to those of gluconic and galactonic. This may indicate that the small and cyclic ANDSA-monosaccharides are more extensively solvated than the larger and linear ANDSA-monosaccharides, which would explain the more pronounced counterion screening of the former than the latter monosaccharides.

In summary, the ionic strength of the separation electrolyte strongly influences the resolution of closely related structures. The proper choice of the ionic strength of the running electrolyte seems to be a good alternative to using complexing buffers such as those based on borate ions for the separation of monosaccharides. The use of high ionic strength electrolyte (i.e., 300 m*M* sodium acetate) was made possible by maintaining the temperature of the separation capillary at 15°C using the cooling system available on the instrument used in this study.

3.2. Sialooligosaccharides derived from gangliosides

The effect of ionic strength on the effective electrophoretic mobility of a group of seven ANDSA labelled sialooligosaccharides derived from gangliosides is shown in Fig. 6. As in the case of the ANDSA-acidic monosaccharides, increasing the ionic strength of the separation electrolyte decreased the electrophoretic mobilities of the ANDSA labelled sialooligosaccharides as a result of screening the charges of these analytes as well as increasing the viscosity of the separation electrolyte. The decrease in the electrophoretic mobility was not the same for the different analytes, thus permitting the modulation of selectivity of the oligosaccharides. As can be seen in Fig. 6, the decrease in the effective electrophoretic mobility as a function of the inverse square root of

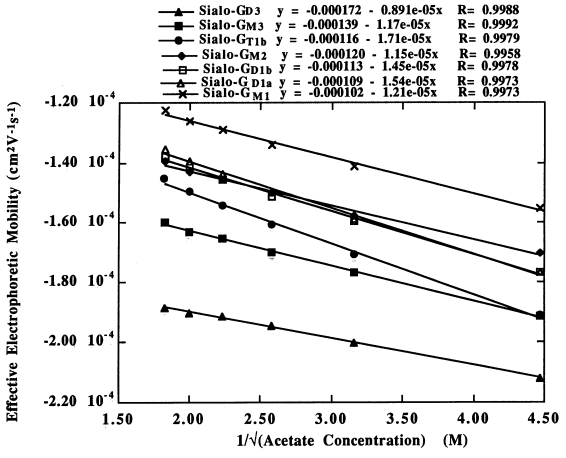

Fig. 6. Plots of the effective electrophoretic mobilities of ANDSA-sialooligosaccharides derived from gangliosides as a function of the reciprocal of the square root of sodium acetate concentration in the running electrolyte. Experimental conditions as in Fig. 3.

sodium acetate concentration is much steeper with ANDSA-trisialooligosaccharides (e.g., the ANDSA derivatives of sialo-G_{T1b} with six negative charges) than with ANDSA-disialooligosaccharides (e.g., the ANDSA derivatives of sialo-G_{D1a}, sialo-G_{D1b} and sialo-G_{D3}, each with four negative charges) and ANDSA-monosialooligosaccharides (e.g., the ANDSA derivatives of sialo-G_{M1}, sialo-G_{M2}, sialo-G_{M3}, each with two negative charges). For the same number of sulfonic acid groups, the decrease in the electrophoretic mobility as a function of the inverse square root of electrolyte concentration is less steeper with unbranched solute (e.g., sialo-G_{D3} and sialo-G_{M3}) than with the branched ones (e.g., sialo-G_{D1a}, sialo-G_{D1b}, see Fig. 1 for structures). The extent of charge screening by electrolyte counterions is more pronounced for solutes whose charges are located on two different parts of the molecule (sialo-G_{D1a} vs. sialo-G_{D1b}).

The increase in the ionic strength of the separation electrolyte strongly influenced the electrophoretic mobilities of the analytes to various extents, thus effecting resolution and selectivity. As the ionic strength was increased, the extent of resolution between the structural isomers namely the ANDSA derivatives of sialo-G_{D1a} and sialo-G_{D1b} was substantially increased from co-migration at 50 m*M* sodium acetate to baseline resolution at 300 m*M* sodium acetate in the running electrolyte (Fig. 7a–c). Also, the migration order and selectivity among the ANDSA derivatives of sialo-G_{M2}, sialo-G_{D1b} and sialo-G_{D1a} were strongly affected by the ionic strength. This is an indication that, at relatively high sodium acetate concentration (i.e., >200 mM) in the running electrolyte, charges on the disialooligosaccharides were screened to an extent that their effective charge became somewhat equivalent to that of the ANDSA derivative of monosialo-G_{M2}. Under these conditions, the migration order of the ANDSA derivatives of sialo-G_{M2}, sialo-G_{D1a} and sialo-G_{D1b} is governed by the branching of the solute molecule. ANDSA-Sialo-G_{M2} being unbranched and small migrated faster, while ANDSA-sialo-G_{D1a} having one bulky ANDSA-NeuAc branching on the Gal-Glc

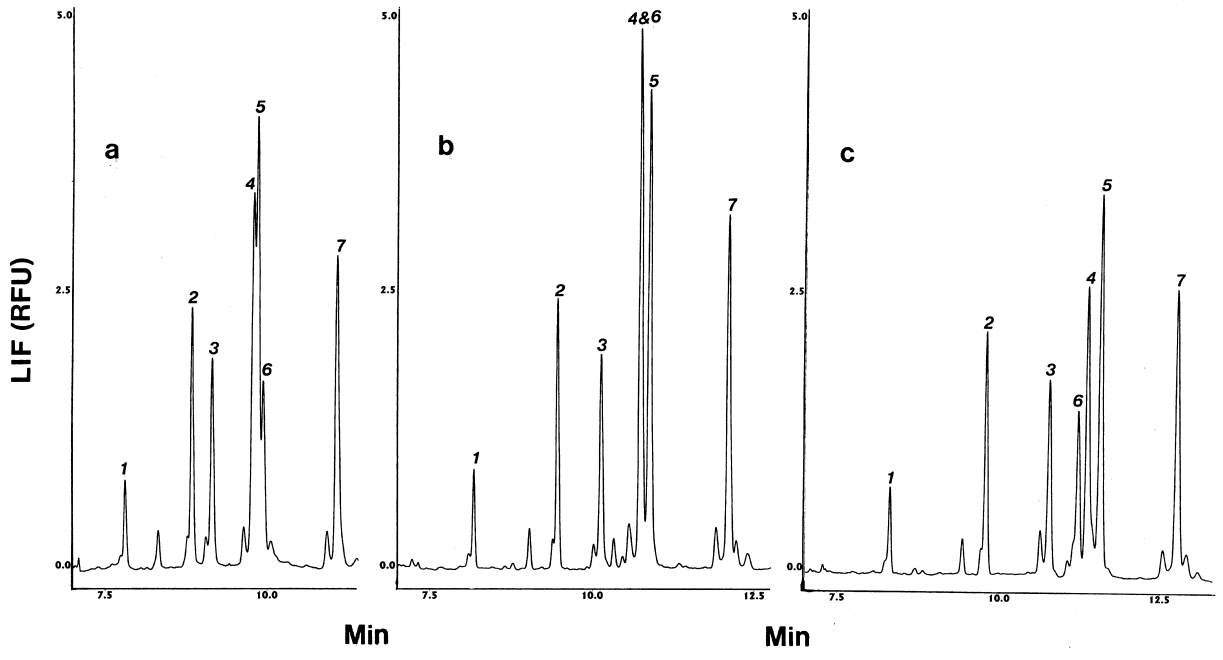

Fig. 7. Electropherograms of ANDSA-sialooligosaccharides derived from gangliosides at various acetate buffer concentration. Conditions: (a) running electrolyte, 100 m*M* sodium acetate, (b) 200 m*M* sodium acetate, (c) and 300 m*M* sodium acetate, pH 5.0. Other conditions as in Fig. 3. Peak assignment: 1, ANDSA-Sialo-G_{D3}; 2, ANDSA-Sialo-G_{M3}; 3, ANDSA-Sialo-G_{T1b}; 4, ANDSA-Sialo-G_{D1a}; 5, ANDSA-Sialo-G_{D1b}; 6, ANDSA-Sialo-G_{M2}; and 7, ANDSA-Sialo-G_{M1}.

migrated second followed by ANDSA-sialo-G_{D1b} which has a branching at the Gal-Glc with two bulky ANDSA-NeuAc moieties.

In this study we have demonstrated that the selectivity in CE can be favorably altered by changing the ionic strength of the separation electrolyte. In other words, varying the ionic strength of the separation electrolyte influenced the electrophoretic mobility of the analyte through the charge-screening effect. The charge-screening effect can modulate resolution and selectivity in a manner as effective as what can be achieved by in situ complexation of sugars with borate-based electrolyte systems.

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