# Capillary zone electrophoresis of linear and branched oligosaccharides☆

## Wassim Nashabeh and Ziad El Rassi*

*Department of Chemistry, Oklahoma State University, Stillwater, OK 74078-0447 (USA)*

## ABSTRACT

The electrophoretic behavior of derivatized linear and branched oligosaccharides from various sources was examined in capillary zone electrophoresis with polyether-coated fused-silica capillaries. Two UV-absorbing (also fluorescent) derivatizing agents (2-amino-pyridine and 6-aminoquinoline) were utilized for the electrophoresis and sensitive detection of neutral oligosaccharides, *e.g.*, N-acetylchitooligosaccharides, high-mannose glycans and xyloglucan oligosaccharides. The oligosaccharides labelled with 6-aminoquinoline yielded eight times higher signal than those tagged with 2-aminopyridine. Plots of logarithmic electrophoretic mobilities of labelled N-acetylchitooligosaccharides with 6-aminoquinoline or 2-aminopyridine *versus* the number of sugar residues in the homologous series yielded straight lines in the size range studied, the slopes of which were independent of the tagging functions. The slopes of these lines are referred to as the N-acetylglucosaminyl group mobility decrement. The oligosaccharides were better resolved in the presence of tetrabutylammonium bromide in the running electrolyte. Furthermore, the electrophoretic mobilities of branched oligo-saccharides were indexed with respect to linear homooligosaccharides, an approach that may prove valuable in correlating and predicting the mobilities of complex oligosaccharides.

## INTRODUCTION

The separation of carbohydrates by electrophoresis has often required the *in situ* conversion of these compounds into charged species. Early investigations on carbohydrate electrophoresis described anionic complexes with sodium borate [1], sulphonated benzene boronic acid [2], sodium germanate [3], sodium stannate [1] or sodium tungstate [4]. Cationic complexes of carbohydrates with lead acetate [5], or cations of the alkali and alkaline earth metals [5] were also described for the electrophoresis of neutral carbohydrates. Furthermore, because of the ionization of the hydroxyl groups of the sugars at high pH, sodium hydroxide was also useful for the electrophoresis of neutral carbohydrates [6]. These buffer systems are expected to find their way to the capillary zone electrophoresis of sugars. In fact, recently capillary zone electrophoresis (CZE) systems based on borate complexes for mono- and oligosaccharides have been described [7,8].

Another difficulty in the analysis of sugars is the lack of chromophores in their structures. This can be overcome by precolumn derivatization [7–11] or by indirect detection [12]. However, a precolumn derivatization that supplies both the charge and the chromophore for the electrophoresis and the sensitive detection, respectively, is preferred.

In recent reports from our laboratory, we have demonstrated the potential of CZE in the separation of 2-aminopyridyl derivatives of maltooligosaccharides [9] and complex-type glycans cleaved from glycoproteins [10]. In the present article, the scope of applications of CZE was extended to the separation and determination of the homologous series of N-acetylchitooligosaccharides, the branched xylo-

glucan oligosaccharides from cotton cell walls, and the high-mannose glycans of ribonuclease B. The electrophoretic mobility of the 2-aminopyridyl derivatives of xyloglucan fragments, was indexed to that of the homologous series, which permitted the evaluation of the contribution of sugar residues and degree of branching to the electrophoretic mobility of the derivatized xyloglucan oligosaccharides. In addition, the potential of 6-aminoquinoline as a new tagging agent that provides both the charge and the center for detection was investigated.

## EXPERIMENTAL

### Instruments

The instrument for capillary electrophoresis was assembled in our laboratory from commercially available components. It comprised two high-voltage power supplies of positive and negative polarity from Glassman High Voltage (Whitehouse Station, NJ, USA) and a Linear (Reno, NV, USA) Model 200 UV–VIS variable-wavelength detector equipped with a cell for on-column capillary detection. The detection wavelength was set at 240 nm for sensing the derivatized oligosaccharides. The electropherograms were recorded with a Shimadzu computing integrator (Columbia, MD, USA) equipped with a floppy disk drive and a cathode-ray tube monitor.

The absorption spectra of the derivatizing agents, *i.e.*, 2-aminopyridine and 6-aminoquinoline were performed on a UV–VIS spectrophotometer (Model UV-160, Shimadzu) by scanning from 200 to 310 nm.

### Capillary columns

Fused-silica capillary columns of 50 $\mu$m I.D. × 365 $\mu$m O.D. were obtained from Polymicro Technology (Phoenix, AZ, USA). All capillaries used in this study were coated in the laboratory with an interlocked polyether coating according to previously described procedures [13]. The running electrolyte was renewed after each run. To ensure reproducible separations the capillary column was flushed successively with fresh buffer, water, methanol, water and again running buffer. The capillary was allowed to equilibrate for 10 min before each injection.

### Reagents and materials

Xyloglucan oligosaccharides were obtained from cotton cell walls as described earlier [14]. They were supplied as 2-aminopyridyl derivatives by Dr. A. Mort from the Department of Biochemistry. N-Acetylchitooligosaccharide standards having a degree of polymerization (DP) from 2 to 6 were from Seikagaku Kogyo (Tokyo, Japan). High-mannose oligostandard, $(GlcNAc)_2$-$Man_5$, was purchased from Dionex (Sunnyvale, CA, USA). Ribonuclease B (RNase B), L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, N-acetylglucosamine (GlcNAc), 2-aminopyridine (2-AP), tris(hydroxymethyl)aminomethane (Tris) and Brij 35 were obtained from Sigma (St. Louis, MO, USA). Peptide-N-glycosidase F (PNGase F) from *Flavobacterium meningosepticum* was obtained from Boehringer Mannheim (Indianapolis, IN, USA). Mercaptoethanol, sodium cyanoborohydride, 6-aminoquinoline (6-AQ), trifluoroacetic acid (TFA) and tetrabutylammonium bromide ($Bu_4N^+$) were from Aldrich (Milwaukee, WI, USA). Reagent-grade sodium phosphate monobasic and dibasic, phosphoric acid, hydrochloric acid, acetic acid, sodium hydroxide, calcium chloride, ethylenediaminetetraacetic acid disodium salt (EDTA), and HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, USA). Deionized water was used to prepare the running electrolyte. All solutions were filtered with 0.2-$\mu$m UniPrep syringeless filters obtained from Genex (Gaithersburg, MD, USA) to avoid column plugging.

### Cleavage of high-mannose glycans

Bovine RNase B was first digested with TPCK-treated trypsin using a 10 m$M$ Tris buffer containing 100 m$M$ ammonium acetate and 0.1 m$M$ calcium chloride, pH 8.3, at a trypsin substrate ratio of 1:100 and a temperature of 37°C [15]. Trypsin was added again after 2 h and the digestion was stopped after a total of 4 h by addition of phosphoric acid. Thereafter, the whole digest was desalted by passing it on a Bakerbond wide-pore octadecyl-silica column (250 × 4.6 mm) equilibrated with water at 0.05% (v/v) TFA. The bound materials were eluted stepwise with acetonitrile–water (80:20, v/v) at 0.05% TFA (v/v). The pooled fraction was evaporated to dryness using a SpeedVac concentrator (Savant,

Farmingdale, NY, USA). The dried materials were then dissolved in 20 m$M$ phosphate buffer containing 2 m$M$ EDTA, 1% (v/v) mercaptoethanol and 0.1% (w/v) Brij 35, pH 7.5. To this solution 3 units of peptide-N-glycosidase F were added, and the incubation was maintained at 37°C for 24 h [16]. Thereafter, the mixture was evaporated to dryness with a Savant SpeedVac concentrator. The dried material containing the cleaved oligosaccharides, the peptide fragments, and other reagents employed in the incubation step was used as is without a sample clean up prior to the derivatization of its oligosaccharide components.

### Derivatization of oligosaccharides

Commercially available N-acetylchitooligosaccharides were tagged with 2-AP or 6-AQ whereas the oligosaccharides cleaved from bovine ribonuclease B were derivatized with 2-AP, at their reducing termini via reductive amination according to the following equations:
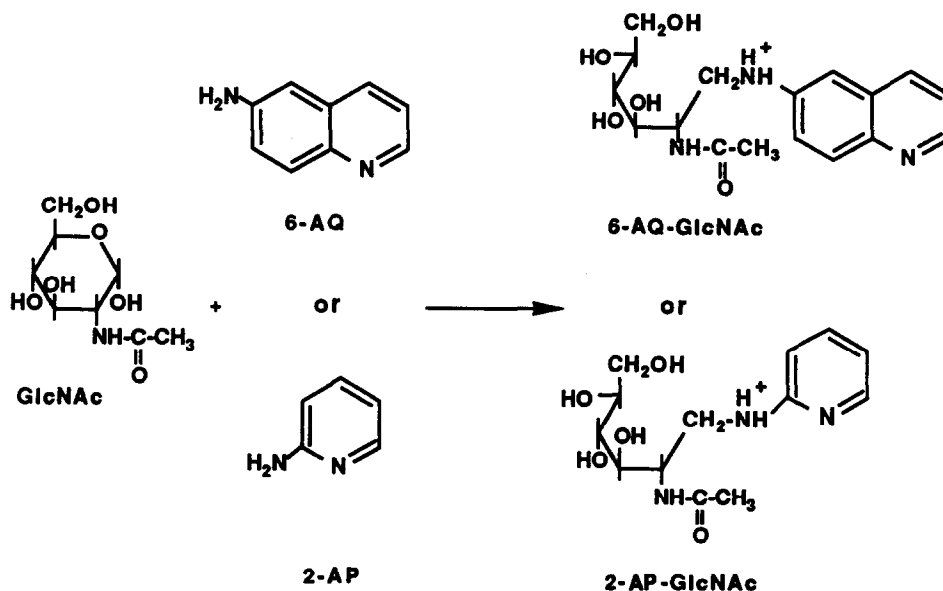


where N-acetylglucosamine is taken as a typical example. To that end a 0.26 $M$ aqueous solution of 2-AP or 6-AQ was titrated to pH 5.8 with glacial acetic acid. Thereafter, sodium cyanoborohydride was added at a concentration of 20 mg/ml just prior to the addition of the carbohydrate. The reaction mixtures were incubated overnight at 70°C. Following, the reaction mixtures containing the derivatized oligosaccharides were evaporated to dryness. The dried materials thus obtained were dissolved in water and then applied to capillary electrophoresis without any sample clean up from excess derivatizing agent and other components of the reaction mixture.

### RESULTS AND DISCUSSION

The electrophoretic behavior of derivatized linear

TABLE I

LOGARITHMIC N-ACETYLGLUCOSAMINYL GROUP MOBILITY DECREMENT, $\delta_{GlcNAc}$, AS ESTIMATED FROM THE SLOPE OF THE LOGARITHMIC ELECTROPHORETIC MOBILITY *VERSUS* THE NUMBER OF GlcNAc RESIDUES IN THE 2-AP-GlcNAc$_n$ HOMOLOGOUS SERIES

Electrolytes: 0.1 $M$ phosphate solution, pH 5.0, at various tetrabutylammonium bromide concentrations.

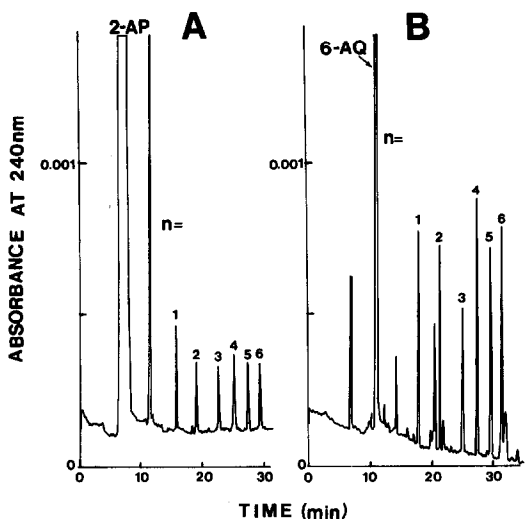| [Bu$_4$N$^+$] (m$M$) | $\delta_{GlcNAc}$ | Correlation coefficient |
|---|---|---|
| 0 | −0.082 | 0.981 |
| 10.0 | −0.105 | 0.990 |
| 30.0 | −0.109 | 0.989 |
| 50.0 | −0.122 | 0.990 |

Fig. 1. Electropherograms of 2-aminopyridyl (A) and 6-amino-quinolyl (B) derivatives of N-acetylchitooligosaccharides. Capillary, fused-silica tube with polyether interlocked coating on the inner walls, 50 cm (to the detection point), 80 cm total length × 50 μm I.D.; electrolyte, 0.1 $M$ phosphate solution containing 50 m$M$ tetrabutylammonium bromide, pH 5.0; running voltage, 18 kV. 2-AP = 2-Aminopyridine; 6-AQ = 6-aminoquinoline.
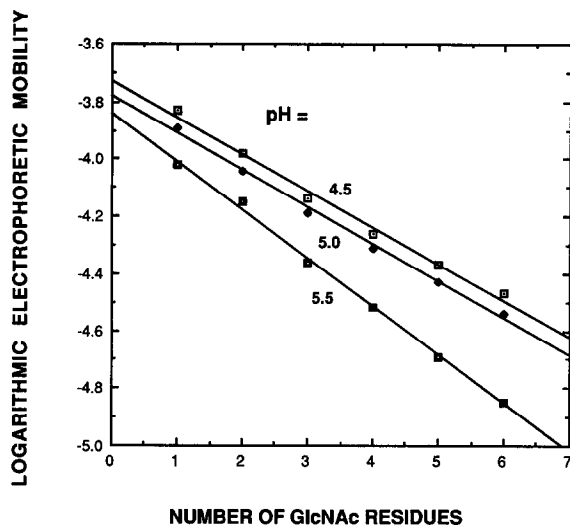


Fig. 2. Plots of logarithmic electrophoretic mobility *versus* the number of GlcNAc residues in the 2-AP-GlcNAc$_n$ homologous series at various pH. Electrolytes, 0.1 $M$ sodium phosphate solutions containing 50 m$M$ tetrabutylammonium bromide. Other conditions as in Fig. 1.

and branched oligosaccharides from various sources was examined using fused-silica capillaries with hydrophilic coating. The results obtained with 2-aminopyridyl derivatives were compared to those of 6-aminoquinolyl derivatives. A mobility indexing system for branched oligosaccharides was established with repect to linear homooligosaccharides.

## CZE of derivatized N-acetylchitooligosaccharides

*N-Acetylglucosaminyl group mobility decrement.* Fig. 1A and B illustrates the high resolution separation of 2-aminopyridyl and 6-aminoquinolyl derivatives of N-acetylchitooligosaccharides, respectively. It was brought about by adding tetrabutylammonium bromide (Bu$_4$N$^+$) to the running electrolyte, a medium that we have reported earlier for the separation of maltooligosaccharides [9] and complex glycans cleaved from glycoproteins [10]. To further characterize the effect of the organic salt on the separation of derivatized oligosaccharides, the 2-aminopyridyl derivatives of N-acetylchitooligosaccharides (2-AP-GlcNAc$_n$) were electrophoresed over a wide range of pH and Bu$_4$N$^+$ concentrations.

Fig. 2 illustrates typical plots of the logarithmic

eletrophoretic mobility, log $\mu$, of 2-AP-GlcNAc$_n$ *versus* the number of N-acetylglucosamine residues (GlcNAc) in the homologous series at different pH. These plots were linear in the size range studied. It has to be noted that the derivatized oligosaccharides carry the same net positive charge, and therefore the charge to size ratio decreases with the number of GlcNAc residues in the homologous series. As can be seen in Fig. 2, the electrophoretic mobility decreased with the pH as the derivatized oligosaccharides become less positively charged. As a result, the slope of the straight lines, which are referred to as the "N-acetylglucosaminyl group mobility decrement", $\delta_{GlcNAc}$, increased in absolute value with increasing the pH in the range studied. However, the resolution between the homologs decreased as the pH increased due to an increase in the electroosmotic flow of the medium with increasing pH.

As expected, the 6-aminoquinolyl derivatives of N-acetylchitooligosaccharides (6-AQ-GlcNAc$_n$) exhibited behavior similar to 2-AP-GlcNAc$_n$. Plots of log $\mu$ *vs.* the number of GlcNAc residues of the homologous series labelled wth 6-AQ, were also straight lines, and their slopes were identical to those

obtained with 2-AP-GlcNAc$_n$ under otherwise identical conditions. This is to say that the $\delta_{GlcNAc}$ is the same regardless of the tagging functions. Note that both derivatizing agents have similar characteristic charges.

To study the effect of Bu$_4$N$^+$ on the electrophoretic behavior of derivatized N-acetylchitooligosaccharides, 2-AP-GlcNAc$_n$ were analyzed in the presence of various amounts of the organic salt in 0.1 $M$ phosphate solutions, pH 5.0. Upon adding the salt to the running electrolyte, the migration time of the homologous series increased monotonically with increasing the salt concentration in the range studied. The plots of log $\mu$ versus the number of GlcNAc residues at different concentrations of Bu$_4$N$^+$ in the running electrolyte were also linear. Their slopes represent $\delta_{GlcNAc}$, and are summarized in Table I. The increase in the values of $\delta_{GlcNAc}$ with increasing Bu$_4$N$^+$ concentration reflects an increase in the resolution between the homologous series. It can be seen in Table I that the addition of only 10 m$M$ of the organic salt to the running electrolyte was enough to yield an absolute increase of about 28% in the value of $\delta_{GlcNAc}$, while 50 m$M$ Bu$_4$N$^+$ exhibited only 48% increase in the absolute value of $\delta_{GlcNAc}$.

To better assess the effect of the organic salt, the electroosmotic flow was measured at each salt concentration using methanol as the inert tracer. Typical results obtained with N-acetylchitohexaose are depicted in Fig. 3 by plots of the overall and electrophoretic mobilities of this sugar as well as the electroosmotic flow versus the concentration of the organic salt in the running electrolyte. As shown in Fig. 3, the decrease in the net mobility of the various homologues could not be only accounted for by the slight decrease in the electroosmotic flow at higher salt concentration, but due to the continuous decrease in the electrophoretic mobilities of the 2-AP-GlcNAc$_n$ with increasing the ionic strength of the running buffer [17–19].

*Comparison of the derivatizing agents.* Although the derivatization with 2-AP is quite reproducible and allows the electrophoresis of carbohydrates by CZE, the 2-aminopyridyl derivatives of mono- and oligosaccharides exhibited limited detection sensitivity in the UV. In search for a more sensitive tagging agent, the performance of 6-AQ, a UV-absorbing and fluorescent tag, was investigated in this study and compared to that of 2-AP using an
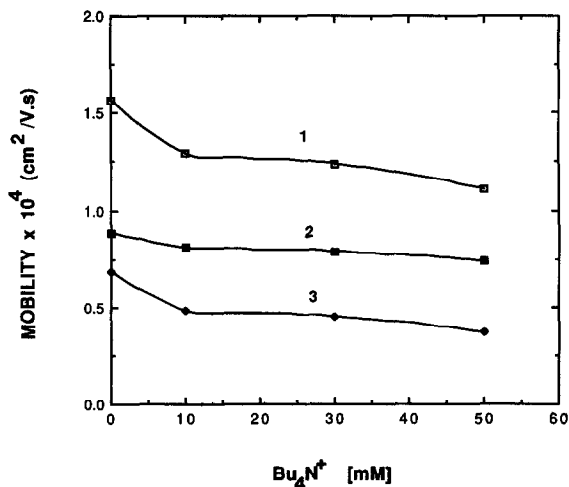


Fig. 3. Plots of overall and electrophoretic mobilities of the 2-aminopyridyl derivative of N-acetylchitohexaose as well as electroosmotic flow at various concentrations of tetrabutylammonium bromide in the running electrolyte, pH 5.0. Curves: 1 = overall; 2 = electroosmotic; 3 = electrophoretic. Other conditions as in Fig. 1.

N-acetylchitooligosaccharides mixture ranging in size from N-acetylglucosamine to N-acetylchitohexaose as model sugar substrates.

Returning to Fig. 1A and B, the 6-aminoquinolyl derivatives of the oligosaccharides (6-AQ-GlcNAc$_n$) exhibited slighty higher migration times than that of 2-AP-GlcNAc$_n$. This is attributed to the lower p$K_a$ value of 6-AQ (p$K_a$ = 5.64) as compared to 2-AP (p$K_a$ = 6.71). Also, 6-AQ has a slightly higher molecular size. As can be noted from Fig. 1A and B, the homologues taggad with 6-AQ showed almost 2 to 3 times higher detection signal than those labelled with 2-AP as estimated from peak height and area calculations from six consecutive runs.

To further characterize the derivatization reactions under investigation, spectral analysis was carried out on both tagging agents and their sugar derivatives. The absorption spectrum of 2-AP (see Experimental) revealed two maxima at 229 and 290 nm with the most intense being at 229 nm, while the 6-AQ spectrum showed an absorption band with a maximum at 242 nm. Due to the difficulty of obtaining the derivatized oligosaccharides in a highly pure form, their absorbance were measured from the electropherograms obtained from various elec-
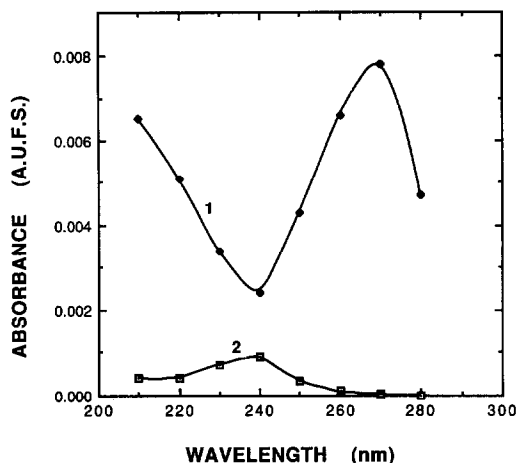
Fig. 4. Plots of absorbance of 6-AQ-GlcNAc$_3$ (1) and 2-AP-GlcNAc$_3$ (2) *versus* the detection wavelength. Conditions are as in Fig. 1, except electrolyte was 0.1 $M$ sodium phosphate solution at pH 5.0.

trophoretic runs. Typical results obtained with 2-AP-GlcNAc$_3$ and 6-AQ-GlcNAc$_3$ are depicted in Fig. 4 by plots of the absorbance at maximum peak heights as a function of the detection wavelength in the range 210 to 280 nm in an increment of 10 nm. The $\lambda_{max}$ for both the 2-aminopyridyl and 6-aminoquinolyl derivatives shifted toward higher wavelengths than the pure derivatizing agents. The $\lambda_{max}$ of 2-AP-GlcNAc$_n$ is around 240 nm and that of 6-AQ-GlcNAc$_n$ is at *ca.* 270 nm. As can be seen in Fig. 4, under the same electrophoretic conditions and in the wavelength range investigated, the 6-aminoquinolyl derivatives exhibited higher absorbance. When compared at their $\lambda_{max}$, 6-AQ-GlcNAc$_n$ yielded a detection signal 8 times higher than that of the sugar tagged with 2-AP. The derivatization procedure described here may prove useful for fluorescence detection. In fact, 6-AQ possess ideal fluorescence properties [20] in the sense that its excitation wavelength (355 nm) is far removed from its emission wavelength (550 nm) and is therefore expected to give low detection limits with fluorescence detector.

## CZE of 2-AP-xyloglucan oligosaccharides

*Mapping.* Xyloglucans are branched oligosaccharides having a backbone identical to that of cellulose [*i.e.*, the (1 → 4) $\beta$-linked D-glucan] with various side chains comprising xylose, galactosyl-

xylose, fucosyl-galactosyl-xylose and seldom arabinosyl-xylose. When digested with cellulase, the backbone of xyloglucans are cleaved after any glucosyl residue with no side chain linked to it. Therefore, the fragments obtained are reflective of xyloglucan branching.

The 2-aminopyridyl derivatives of the xyloglucan fragments (2-AP-XG) obtained by cellulase digestion were first analyzed by reversed-phase chromatography as reported earlier [14]. The collected fractions the structures of which were assessed tentatively by liquid secondary-ion mass spectrometry and previously proven structures were then analyzed by CZE using the electrophoretic system described for the homologous series. Fig. 5 is a typical electropherogram of a mixture reconstituted by mixing aliquots from selected reversed-phase chromatography fractions. It was performed on a
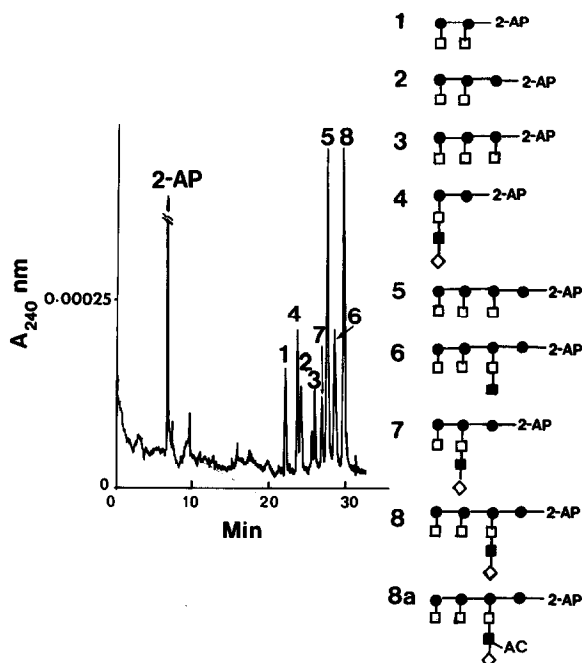


Fig. 5. Capillary zone electrophoresis mapping of the 2-aminopyridyl derivatives of xyloglucan oligosaccharides reconstituted by mixing aliquots from fractions collected during various chromatographic runs. Capillary, fused-silica tube with polyether interlocked coating on the inner walls, 50 cm (to the detection point), 80 cm total length × 50 $\mu$m I.D.; electrolyte, 0.1 M sodium phosphate solution containing 50 m$M$ tetrabutylammonium bromide, pH 4.75; running voltage, 20 kV. Symbols: ● = glucose; □ = xylose; ■ = galactose; ◇ = fucose; AC = acetyl group.

fused-silica capillary with a polyether interlocked coating using 0.1 $M$ phosphate containing 50 m$M$ $Bu_4N^+$ at pH 4.75. As expected the elution pattern in CZE was different from that in reversed-phase chromatography. The peak numbering on the electropherogram (see Fig. 5) reflects the elution order obtained in reversed-phase chromatography. In CZE the elution order was mainly governed by the number of sugar residues and the degree of branching, whereas in reversed-phase chromatography the elution order was mainly influenced by the size of the oligosaccharide and the hydrophobic character of the sugar residues. For instance, fragment 4 which is smaller in size than fragment 3 was more retarded on the reversed-phase chromatography column. This may be attributed to the presence of fucosyl residue in structure 3, which is more hydrophobic than any other sugar residues in the molecule. The same reasoning can explain the elution order for fragments 6 and 7. In CZE, due to the fact that all 2-AP-XG fragments possess the same charge they migrated in the order of increasing size as the charge to mass ratio decreased. However, for the same number of residues but with slight differences in molecular weight the less branched oligosaccharides eluted earlier than the more branched one. In fact, structure 4, which has a slightly higher molecular weight than structure 2 eluted first. This may be

explained by the fact that structure 2 is doubly branched as opposed to fragment 4 which is singly branched. The same behavior was observed for fragments 7 (doubly branched) and 5 (triply branched). Thus, as the extent of oligosaccharide branching increased, the electrophoretic mobility decreased.

Fig. 6 illustrates the high separation efficiency of acetylated xyloglucan oligosaccharides generated by a slightly different method [14] than that used to generate the fragments used in Fig. 5. The average plate count per meter was about 225 000 as calculated from the two major fragments 5 and 8. Both structures 8 and 8a are nonasaccharides with the same extent of branching. The only difference is that fragment 8a has an acetyl substitution at the galactosyl residue that is not present in structure 8. This once more shows the high resolving power of CZE in recognizing small differences between the various xyloglucan fragments. The peaks labelled with asterisks may be attributed to other oligosaccharides present in the mixture.

*Mobility indices.* To interpret the electrophoretic behavior of the various 2-AP-XG and to quantitatively describe the effects of the various sugar residues on the electrophoretic mobility, we have attempted the indexing of the electrophoretic mobilities of the branched xyloglucan oligosaccharides with respect to the linear 2-AP-GlcNAc$_n$. In this regard, the mobility indices, M.I., of the 2-AP-XG fragments were calculated using the following equation:

$$M.I. = 100n + 100 \left( \frac{\log \mu_s - \log \mu_{n+1}}{\log \mu_n - \log \mu_{n+1}} \right)$$

where $\mu_s$ is the electrophoretic mobility of the 2-AP-XG solute, $\mu_n$ and $\mu_{n+1}$ are the electrophoretic mobilities for the two homologues with $n$ and $n + 1$ repetitive units which eluted before and after the xyloglucan fragment, respectively. This indexing is similar to that of Kováts retention indices for gas–liquid chromatography [21]. Table II compiles the values of mobility indices of selected 2-AP-XG fragments with respect to the homologous series, 2-AP-GlcNAc$_n$. In this table the mobility indices, M.I. were averaged over the pH range 4.5 to 5.25 and for $Bu_4N^+$ concentrations from 0 to 50 m$M$. The difference in mobility indices between an adjacent pair of 2-AP-XG fragments, $\Delta$M.I., is referred
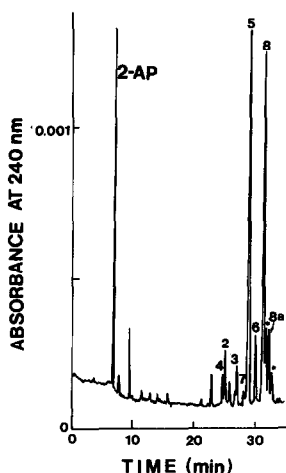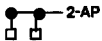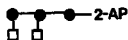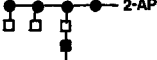
Fig. 6. Capillary zone electrophoresis mapping of 2-aminopyridyl derivatives of acetylated xyloglucan oligosaccharides from cotton cell walls. For fragment structures, see Fig. 5. Conditions as in Fig. 5.

TABLE II

MOBILITY INDICES (M.I.) AND GROUP MOBILITY INDEX DECREMENTS ($\Delta$M.I.) OF VARIOUS 2-AP-XG FRAGMENTS WITH RESPECT TO 2-AP-GlcNAc$_n$

See text for calculations. Group I, average values obtained with 0.1 $M$ sodium phosphate solutions, pH 5.0, at 0, 10, 30 or 50 m$M$ tetrabutylammonium bromide; group II, average values obtained with 0.1 $M$ sodium phosphate solutions containing 50 m$M$ tetrabutylammonium bromide at pH 4.50, 4.75, 5.00 or 5.25; group III, overall average. Symbols: ● = glucose; □ = xylose; ■ = galactose; ◇ = fucose.

| Xyloglucan | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| | M.I. | $\Delta$M.I. | M.I. | $\Delta$M.I. | M.I. | $\Delta$M.I. |
| (structure 1) | 267.5 ± 5.6 | 51.5 ± 4.0 | 277.0 ± 7.8 | 53.5 ± 4.0 | 272.3 ± 4.8 | 52.5 ± 2.8 |
| (structure 2) | 319.0 ± 9.0 | 57.1 ± 1.2 | 330.5 ± 10.9 | 53.7 ± 3.0 | 324.8 ± 7.1 | 55.4 ± 1.6 |
| (structure 3) | 376.1 ± 9.0 | 53.3 ± 4.7 | 384.2 ± 10.0 | 54.6 ± 5.2 | 380.2 ± 6.7 | 54.0 ± 3.5 |
| (structure 4) | 429.4 ± 14.0 | 37.4 ± 4.1 | 438.8 ± 16.0 | 39.5 ± 5.0 | 434.1 ± 10.6 | 38.4 ± 3.2 |
| (structure 5) | 466.8 ± 18.0 | 37.9 ± 4.0 | 478.3 ± 20.0 | 36.8 ± 5.6 | 472.5 ± 13.5 | 37.4 ± 3.4 |
| (structure 6) | 504.7 ± 20.0 | | 515.1 ± 20.0 | | 509.8 ± 14.1 | |

to as the group mobility index decrement contributed by a sugar residue added to a parent oligosaccharide molecule. This value was practically the same over the pH range or salt concentrations studied for a given pair of derivatized xyloglucan fragments. Based on these findings, the M.I.s were averaged as indicated in group III in Table II. As can be seen, the xyloglucan fragment 1 eluted between the homologues of DP 2 and 3, respectively. Fragment 2, which has an additional glucosyl residue in the backbone chain of the oligosaccharide, exhibited a mobility index decrement of 52 and eluted between the homologues of DP 3 and 4, respectively. The presence of a xylosyl residue in structure 3 showed a similar change in the mobility index decrement. Furthermore, the addition of a glucosyl unit to the linear core chain imparted an identical increment in the migration time and xyloglucan fragment 5 eluted between the homologues of DP 4 and 5. It is

therefore wise to say that a glucosyl residue in the backbone of the xyloglucan behaves as one half a GlcNAc residue in terms of its contribution to the eletrophoretic mobility of the 2-AP-XG. The same can be stated about adding a xylosyl residue at the glucose loci. However, the addition of a galactosyl residue to an already branched xylosyl residue exhibited less retardation (almost 70%; see mobility index decrements in Table II) than the addition of a glucosyl or xylosyl unit to the backbone of the xyloglucan oligosaccharide. The same observation can be made about adding a fucosyl residue to a branched galactosyl residue. Thus, as the molecule becomes more branched, the addition of a sugar residue does impart a slightly less decrease in its mobility. Such indexing may prove valuable in correlating and predicting the mobilities of oligosaccharides.
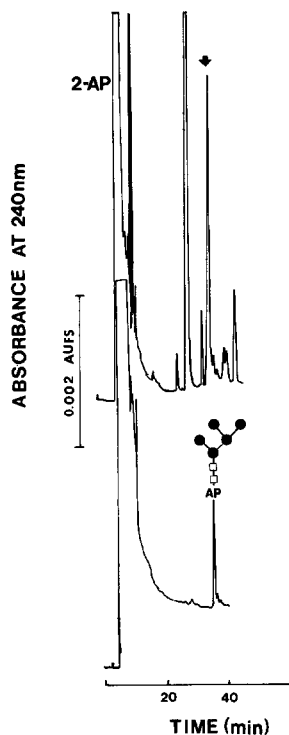
Fig. 7. Capillary zone electrophoresis mapping of 2-aminopyridyl derivatives of high mannose oligosaccharides cleaved from bovine ribonuclease B (top electropherogram), and of 2-AP-$(GlcNAc)_2$-$Man_5$ standard (lower electropherogram). Symbols: □ = GlcNAc; ● = mannose. Conditions as in Fig. 1.

## CZE mapping of oligosaccharide chains from ribonuclease B

To further illustrate the resolving power of the electrophoretic system under investigation, high-mannose glycans were analyzed by CZE. In this regard, oligosaccharides from bovine RNase B were cleaved from the tryptic digest of the protein using peptide-N-glycosidase F, an endoglycosidase that cleaves all types of N-linked oligosaccharide chains between the asparagine and the carbohydrate units. The CZE mapping of the 2-aminopyridyl derivatives of these oligosaccharides is portrayed in Fig. 7. It was performed on a capillary with a polyether interlocked coating using 0.1 $M$ phosphate solution, pH 5.0, containing 50 m$M$ tetrabutylammonium bromide as the running electrolyte. As can be seen in Fig. 7, the peak labelled with an arrow was identified as $(GlcNAc)_2$-$Man_5$ using an oligosaccharide standard. In fact, ribonuclease B is known as a source of

high mannose oligosaccharides with only one glycosylation site. This results in heterogeneous populations of structurally related oligosaccharides which renders RNase B, as most other glycoproteins, highly microheterogeneous. This may expain the presence of several peaks in the CZE map besides that of $(GlcNAc)_2$-$Man_5$ oligosaccharide which, according to Liang et al. [22], is the most predominant carbohydrate moiety of bovine ribonuclease B.

## REFERENCES

1 E. M. Lees and H. Weigel, J. Chromatogr., 16 (1964) 360.
2 P. J. Garegg and B. Lindberg, Acta Chem. Scand., 15 (1961) 1913.
3 B. Lindberg and B. Swan, Acta Chem. Scand., 14 (1960) 1043.
4 M. Weigel, Adv. Carbohydr. Chem., 18 (1963) 61.
5 J. L. Frahn and J. A. Mills, Aust. J. Chem., 12 (1959) 65.
6 G. Zweig and J. R. Whitaker, Paper Chromatography and Electrophoresis, Academic Press, New York, 1967, p. 233.
7 S. Honda, S. Iwase, A. Makino and S. Fujiwara, Anal. Biochem., 176 (1989) 72.
8 S. Honda, A. Makino, S. Suzuki and K. Kakehi, Anal. Biochem., 191 (1990) 228.
9 W. Nashabeh and Z. El Rassi, J. Chromatogr., 514 (1990) 57.
10 W. Nashabeh and Z. El Rassi, J. Chromatogr., 536 (1991) 31.
11 J. Liu, O. Shirota and M. Novotny, Anal. Chem., 63 (1991) 413.
12 E. S. Yeung and W. G. Kuhr, Anal. Chem., 63 (1991) 275A.
13 W. Nashabeh and Z. El Rassi, J. Chromatogr., 559 (1991) 367.
14 Z. El Rassi, D. Tedford, J. An and A. Mort, Carbohydr. Res., 215 (1991) 25.
15 C. B. Kasper, in S. B. Needleman (Editor), Protein Sequence Determination, Springer, Berlin, New York, 1975, p. 114.
16 A. L. Tarentino, C. M. Gomez and T. H. Plummer, Biochemistry, 24 (1985) 4665.
17 C. J. O. R. Morris and P. Morris, Separation Methods in Biochemistry, Wiley, New York, 1976, p. 711.
18 K. D. Altria and C. F. Simpson, Chromatographia, 24 (1987) 527.
19 H. I. Issaq, I. Z. Atamna, G. M. Muschik and G. M. Janini, Chromatographia, 32 (1991) 155.
20 P. J. Brynes, P. Bevilacqua and A. Green, Anal. Biochem., 116 (1981) 408.
21 E. sz. Kováts, Adv. Chromatogr., 1 (1965) 229.
22 C. J. Liang, K. Yamashita and A. Kobata, J. Biochem., 88 (1980) 51.