[16] Capillary Electrophoresis of Oligosaccharides

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Introduction

Electrophoresis is a method of separation that relies on the migration of charged substances in a conducting solution under the influence of an applied electrical field. It is the principal tool for the analysis of peptides and nucleic acids and has been applied to the analysis of oligosaccharides as well.

Charged molecules migrate in an applied electric field at neutral pH. There are many charged carbohydrates that can be directly analyzed by electrophoresis. Ionic carbohydrates include (1) acidic polysaccharides such as pectin and alginate acid, (2) glycosaminoglycans, including hyaluronic acid, chondroitin sulfates, dermatan sulfates, heparan sulfate, heparin, and keratan sulfate, and (3) acidic oligosaccharides, including sialylated, sulfated, and phosphorylated oligosaccharides.

Because electrophoresis generally involves migration of charged species in an electric field, it is not immediately apparent how this technique applies to neutral carbohydrates. Neutral sugars can be given charge by
simply adjusting the pH of their environment, thus most show mobility at high pH. Alternatively, neutral sugars can be given a charge through complexation. Borate ions complex with the vicinal hydroxyl groups of sugars, forming anionic species that can be separated by electrophoresis.1,2 Steric hindrance by carbohydrate side chains and the competitive interaction with other ligands may alter the formation of complexes and thereby provide a wide range of saccharide mobilities. Neutral carbohydrates can also be given charge by their conjugation to a charged species. In addition to ensuring their mobility in an electric field, such derivatization reactions can enhance carbohydrate detection.

Despite its relative ease of use and widespread availability, gel electrophoresis has certain limitations, particularly in sample quantitation and ease of automation. Capillary electrophoresis (CE)3-5 can overcome these limitations but has only recently been used for analyzing carbohydrates. The most common type of CE is capillary zone electrophoresis (CZE). This rapid, high-resolution, sensitive method is based primarily on the differential migration of solutes through a narrow-bore fused silica capillary.3 This chapter focuses on the analysis of both undervatized and derivatized oligosaccharides by CZE in unmodified, unfilled fused silica capillaries. Capillary zone electrophoresis is not generally useful for preparative applications.

Background

Capillary zone electrophoresis is a fast and simple method that represents a powerful tool for analysis and separation of acidic saccharides, as first demonstrated independently by Al-Hakim and Linhardt6 and Carney and Osborne.7 Capillary zone electrophoresis was used to analyze disaccharides derived from chondroitin sulfates, dermatan sulfate, and hyaluronic acid (Fig. 1). These disaccharides were prepared through the action of a lyase that introduces an unsaturated uronic acid having an ultraviolet absorbance at the nonreducing end of the oligosaccharide. These disaccharides had a net charge of -1 to -4 and were resolved primarily on the basis of net charge and secondarily on the basis of charge distribution. The sensitivity of detection of this method surpassed all other

analytical methods for these compounds, permitting the detection of 500 amol. Separation of chondroitin disaccharides in the presence of sodium dodecyl sulfate (SDS) gave improved peak shape and resolution. Ampofo et al. extended this method by separating eight disaccharides prepared from heparin, heparan sulfate, and derivatized heparins (Fig. 1). The effect of capillary length, buffer prepared in $^3$H$_2$O, and SDS in the absence of buffer on peak resolution was also examined.

Sialylated oligosaccharides released from $\alpha$-acid glycoprotein by hydrazinolysis have been analyzed by CZE by Hermentin et al. After

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release, the N-glycans were fractionated by chromatography on the basis of charge. The total N-glycans as well as fractions containing a net charge of $-1$ to $-5$ were analyzed in buffer at neutral pH. These oligosaccharides were detected without derivatization by monitoring absorbance at 190 nm.

Most of carbohydrate analysis using CE has relied on separation of derivatized sugars in unfilled, undervatizated, fused silica capillaries, and detection has been by ultraviolet absorbance. Honda et al.$^{11}$ first demonstrated that carbohydrates could be analyzed by CZE. Reducing monosaccharides, converted to N-2-pyridylglycamines by reductive amination (Fig. 2a), were separated by using a capillary tube of fused silica containing basic borate buffer as carrier. The anionic borate complexes facilitated separation and the ultraviolet absorbance of the derivatized sugars permitted the detection of picomole quantities. This method was applied to the determination of the monosaccharide composition of various oligosaccharides and polysaccharides, including lactose, melibiose, rutin, digitonin, and arabic gum.

Nashabeh and El Rassi$^{12}$ later reported conditions for the separation of pyridylamino derivatives of maltooligosaccharides in the absence of borate ion. Nashabeh and El Rassi$^{13}$ have used this method to separate sialylated oligosaccharides in a derivatized capillary. High separation efficiencies were obtained in phosphate buffer at acidic pH (3.0–4.5). Under these conditions the N-2-pyridylglycamine derivatives (Fig. 2a) are protonated and carry a positive charge. The inclusion of small amounts of tetrabutylammonium bromide in the electrolyte facilitates this separation at pH 5.0 and gives higher separation efficiency. The oligosaccharides eluted in

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the order of increasing size, with mobility being a linear function of the number of glucose residues in the homologous series. These results were consistent with the independent study of Honda et al.,\textsuperscript{14} which extended application of CZE to oligosaccharides derived from glycoproteins. Pyridylaminate derivatives of oligosaccharides released from ovalbumin with hydrazine were analyzed by CZE with fluorescence detection. By using polyacrylamide-coated capillary tubes and phosphate buffer (pH 2.5), both ovalbumin oligosaccharide derivatives and isomaltotriose derivatives [up to a degree of polymerization (DP) of 20] were separated in order of decreasing size. Each oligosaccharide derivative had a single protonated amino group at pH 2.5 that facilitated the separation.

A major limitation of using CZE for carbohydrate analysis has been the absence of sensitive detection methods. Ultraviolet detection has been used for both underivatized and derivatized carbohydrates, but it has limited detection sensitivity. In contrast, fluorescence detection is easily adapted for use in CE and a number of fluorophores have been introduced into the carbohydrate molecules for on-column detection. Lee et al.\textsuperscript{15} first described the use of a charged fluorescent tag, 7-amino-1,3-naphthalenedisulfonic acid (Fig. 2b), for the analysis of carbohydrates by gel electrophoresis. This method was extended to CZE when an approach was developed to examine the action pattern of chitinase on fluorescent conjugates of chitooligosaccharides.\textsuperscript{16} Lee et al.\textsuperscript{17} also demonstrated the usefulness of CZE for measuring fucosyltransferase activity. Capillary zone electrophoresis measures fluorescent conjugates with a sensitivity of 80 fmol.\textsuperscript{17}

Laser sources are capable of focusing light in small capillaries and thus give increased sensitivity of detection. Liu et al.\textsuperscript{18} have employed fluorogenic reagents for laser-induced fluorescence measurements of carbohydrates. By using 3-(4-carboxybenzoyl)-2-quinoline carboxaldehyde as a precolumn derivatization agent for various amino sugars (degradation products of chitosan and a glycoprotein), highly fluorescent isoindole derivatives were determined by CZE at attomole levels.

A wide variety of compounds has been determined by indirect detection without the need for chemical derivatization.\textsuperscript{19} This method relies on the physical displacement of a chromophore or fluorophore in the electro-

lyte by an analyte of similar charge. This results in negative peaks in the electropherogram, due to the absence of fluorophore, and is independent of the spectral characteristics of the analyte. Indirect detection with fluorescein and coumarin fluorophores allows the detection of attomole amounts. Garner and Yeung\textsuperscript{20} extended the use of indirect fluorescence detection for CZE to the visible region, permitting the use of visible optics and more powerful visible-light sources. Glucose, fructose, and sucrose were separated by CZE without prior derivatization at a high pH (resulting in the ionization of the sugar hydroxyl groups). Coumarin, which works well with a 442-nm helium–cadmium laser, was used in the detection of this sugar mixture.

Principle of Method

Capillary zone electrophoresis relies on simple instrumentation that includes a high-voltage supply, a capillary column, and a detector (Fig. 3). The capillary column invariably carries a negative charge on its inner surface because the silanol groups of the uncoated capillary ionize above pH 3. Positive ions in the buffer electrolyte are attracted to the negatively charged surface of the capillary. Under high voltage, the positive buffer ions migrate toward the cathode, creating a bulk flow of electrolyte called the electroosmotic flow. If a sample containing both ionic and nonionic solutes is introduced into the capillary at the anode side, all the components are carried in the electroosmotic flow to the cathode. The positive species elute first, followed by the neutral, and finally by the negative

species, thus effecting a separation (Fig. 4). To optimize separation, the electroosmotic flow is adjusted either by altering buffer composition and pH or by chemically derivatizing the capillary wall. Micellar electrokinetic capillary electrophoresis (MECE) exploits a different separation principle. The addition of sodium dodecyl sulfate (SDS) to the electrolyte buffer results in partitioning of the analyte between the micelle and the surrounding aqueous phase. Separation is accomplished through differential migration of the phases.

Procedure

**Instrumentation**

There are a variety of different instruments that are commercially available. In addition, it is possible to build an instrument from the component parts. All CE instruments are composed of a high-voltage power supply, buffer reservoirs, a capillary, and a detector (Fig. 3). Most commercial instruments also include a sampler/injector and a programmable computer for automated analysis and data processing.

**Preparing Capillary**

The capillary column is typically made of fused silica, externally coated with a 20- to 75-μm i.d. and a 300- to 500-μm o.d., and is usually available in long rolls. It is first cut to the desired length (usually between 0.2 to
1 m), and the external coating is removed from the area where the capillary passes through the detector. The capillary tube is activated by washing extensively with 5 vol each of 0.1 M phosphoric acid, 0.5 M sodium hydroxide, deionized water, and operating buffer and inserted into the machine.

**Preparing Buffer**

The buffer to be used must first be carefully selected. Not all buffers support electroosmotic flow in a capillary. The instruction manual accompanying each instrument usually suggests buffers that can be used for different CZE applications. The buffer should be selected, carefully prepared, and its pH adjusted. Finally, the buffer must be filtered (through a 0.2-μm pore size syringe filter) to remove particles that can clog the narrow capillary and then thoroughly degassed. It is best to prepare fresh buffer each day.

**Preparing Sample**

The sample must be filtered (through a 0.2-μm pore size syringe filter) or centrifuged before analysis to remove particulates. The sample should be at a high concentration, typically between 100 μg/ml and 10 mg/ml (for a carbohydrate of \( M_r \) 500–2500). The sample should be free of salt and other contaminants that could interfere with either electrophoresis or sample detection.

**Injecting Sample**

There are a number of different methods that can be used to inject a sample into the capillary column for analysis. Gravity injection is an unbiased method of introducing both charged and neutral analytes. Typically, gravity injection that relies on capillary action is used. The amount of sample injected can be calculated from the difference in height between the ends of the capillary, the internal diameter of the capillary, and the length of time the elevated end of the capillary is immersed in the sample. This injection method is also referred to as vacuum injection or injection by hydrostatic pressure. Other methods of injection include electroinjection and syringe injection. Electroinjection is biased toward either anionic or cationic species (depending on the direction of current flow), making quantitation difficult. Typically only 1 to 100 nl of sample is injected for each analysis. The volume of analyte injected depends on the capillary volume, the sample concentration, and the detector sensitivity. Typically, an injection volume of >10% of the capillary volume leads to a loss of resolution.
Electrophoresis Conditions

A high voltage, typically ranging from 10 to 20 kV, is used in CZE. The length of time required for each analysis is usually from 2 to 90 min, depending on capillary length, voltage, buffer, and the net charge of the sample. A constant voltage is most commonly used; however, voltage gradients can be used to improve resolution or to minimize analysis times. Electrophoresis can also be done under constant power or constant current.

Detection

Only absorbance and fluorescence detectors are commercially available. The absorbance detectors are either fixed wavelength detectors or variable wavelength detectors. The fluorescence detectors require filters of the appropriate wavelength. A single-wavelength laser-induced fluorescence detector has become available.

Specific Applications

Analysis of Underivatized Carbohydrates

Glycosaminoglycans and Derivatives

Glycosaminoglycans (GAGs) are highly charged, linear, sulfated polysaccharides that can be broken down with polysaccharide lyases into acidic oligosaccharides that absorb at 232 nm. Disaccharides 1–17 (Fig. 1) can be prepared directly from glycosaminoglycans, including hyaluronic acid, chondroitin sulfate and dermatan sulfate, heparin, heparan sulfate, or chemically modified heparin, with polysaccharide lyases [from Seikagaku America, Inc. (Rockville, MD), Grampian Enzymes (Aberdeen, Scotland), and Sigma Chemical (St. Louis, MO)] and purified by strong anion-exchange high-performance liquid chromatography (HPLC). Alternatively, these disaccharide standards are now available from these same companies.

1. Disaccharide standards are accurately weighed, and a 10-mg/ml stock solution of each is prepared in distilled, deionized water. Portions of these stock solutions are mixed together to prepare standard mixtures.

and for coinjection studies. These standard solutions are stored frozen at −70°C.

2. Glycosaminoglycan (10 μg/μl) is treated with the appropriate lyase (2 mU of enzyme/100 μg of substrate) in 5 mM sodium phosphate buffer at pH 7.0 for 12 hr at 30°C.

3. A CE system equipped with an ultraviolet detector set at 232 nm is used for this analysis. Separation uses a fused silica capillary column having an effective length of 68 cm (distance between injector and detector). The buffer used is 10 mM sodium borate containing 50 mM boric acid, having a pH of 8.5, or 10 mM sodium borate and 50 mM SDS, having a pH of 8.5.

4. The sample is injected by gravity injection (1 nl) at the anodic side (positive electrode), and electrophoresis is performed for 0.5 to 1 hr at 10 kV, using the operating buffer. The temperature is maintained at 30°C. A typical electropherogram is shown in Fig. 5.
Comments on Method. A simple mixture containing single disaccharides having a net charge of $-1$ to $-4$ can be used to examine the effect of voltage, capillary, buffer, and pH on migration time. A doubling of the voltage, from 10 to 20 kV, will reduce the migration time by 50% but decreases resolution. The replacement of one capillary tube for a second, identically prepared capillary will only slightly ($\pm$5%) affect the migration times. The use of phosphate buffer (pH 8.8), in place of borate buffer, will increase migration time and decrease resolution. Acidification of the borate buffer to pH 5, to decrease complexation, surprisingly increases disaccharide migration times.

Lengthening the capillary above 70 cm results in only a modest improvement in resolution but increases analysis time. The introduction of 50 mM SDS\(^8\) in the presence or absence of borate buffer can improve resolution but results in a slightly decreased sensitivity. Under optimum conditions, the detection sensitivity of this method is 500 amol to 1 fmol.

The disaccharide composition of a glycosaminoglycan can be analyzed by this method. Oligosaccharides of net charge $>-4$ have similar migration times under the conditions described, thus glycosaminoglycan should be completely converted to disaccharides with a mixture of lyases.\(^8\) These analyses required 15 ng of polysaccharide and a 20-min analysis time. Full quantitation of glycosaminoglycan may require the use of multiple sets of buffer conditions.

Sialylated Oligosaccharides

$\alpha_1$-Acid glycoprotein contains a number of different N-linked sialylated oligosaccharides. These oligosaccharides can be released by hydrazinolysis or by using PNGase F to obtain an N-glycan pool. These acidic oligosaccharides can be fractionated by Mono Q FPLC (fast-protein liquid chromatography, Pharmacia, Piscataway, NJ) ion-exchange chromatography to obtain five fractions containing oligosaccharides with net charge of $-1$ to $-5$.\(^9,10\)

1. Oligosaccharide Mono Q fractions prepared from $\alpha_1$-acid glycoprotein are dissolved in distilled water at approximately 2 mg/ml and stored frozen.

2. A CE system equipped with an ultraviolet detector set at 190 nm is used for the analysis. Separation is in a 100-cm fused silica capillary column. The buffer is 80 mM ammonium sulfate, 20 mM sodium phosphate, 2 mM diaminobutane, adjusted with phosphoric acid to pH 7.

3. The sample is injected by vacuum injection (5 nl) at the anodic side (positive electrode), and electrophoresis is performed for 1 hr at 20 kV. The temperature is maintained at 30°.
Comments on Method. The method of Hermentin and co-workers\(^9,10\) described earlier is one of the most general methods for the analysis of sialylated, glycoprotein-derived oligosaccharides. The most important feature of this method is that no derivatization is required for the detection of femtomole amounts of N-glycan by absorbance at 190 nm. The analysis of \(\alpha\)-acid glycoprotein-derived oligosaccharides as pyridyliaminated derivatives by Nashabeh and El Rassi\(^11\) resulted in only 6 major structures, whereas the analysis described above facilitated the detection of 40 different structures. Whether differences in the observed complexity of the analyte are due to the superior resolution of this method or simply to differences in the heterogeneity of the starting glycoprotein remains to be established.

Indirect Fluorescence Detection

Simple underivatized monosaccharides, disaccharides, and trisaccharides can be analyzed at elevated pH and detected by indirect fluorescence detection.\(^20\)

1. Saccharide samples are analyzed in 10 mM sodium bicarbonate buffer containing 1 mM coumarin adjusted to pH 11.5 with sodium hydroxide.\(^20\)

2. Separations is in a 90-cm fused silica capillary column driven by the ionization of the sugar hydroxyl groups at high pH.

3. Injection of 640 fmol at the anodic side is followed by indirect fluorescence detection with a helium–cadmium laser operating at 442 nm.

Comments on Method. This method avoids the requirement of derivatization by using a high pH to drive the separation and an indirect method of detection. As more complex mixtures of sugars are analyzed, the resolution achieved at high pH may be insufficient for adequate separation. Additionally, high pH is known to result in oligosaccharide peeling\(^22\) and other reactions that damage sugar fine structure. Indirect detection of oligosaccharides can also be performed in a standard fluorescence detector.\(^24\)

Analysis of Derivatized Carbohydrates

Conjugation with Neutral Tag

Oligosaccharides that are tagged with 2-aminopyridine (Fig. 2a) are neutral derivatives and can be effectively analyzed by CZE only if they are first given a charge. Two approaches are typically used to accomplish


this. In the first, the pyridylamino derivatives of neutral oligosaccharides are converted in situ to anionic borate complexes by using borate buffer at high pH. In the second, these pyridylamino derivatives are given a positive charge by protonation in an acidic (low-pH) buffer.

2-Aminopyridine Reagent Solution. To a mixture of methanol (350 µl) and glacial acetic acid (80 µl), 2-aminopyridine (184 mg) and sodium cyanoborohydride (35 mg) are added. The solution is mixed in vortexer and used immediately.

1. Preparation of oligosaccharide conjugates is accomplished by adding 40 µl of the 2-aminopyridine reagent solution to 0.1–1 mg of oligosaccharide in 10 µl of water.14,25 The mixture is heated for 15 hr at 80°.

2. To purify the oligosaccharide derivatives, the reaction is first cooled and then applied to a Sephadex G-15 column (1 × 50 cm), followed by elution with 15 mM ammonium acetate in 25% aqueous methanol. The column is monitored by fluorescence (λ_ex = 316 nm, λ_em = 395 nm). The void and included volumes, containing derivatized oligosaccharide, are combined and dried in vacuo. The partially purified product is dissolved in water and applied to a Dowex 50-X2H⁺ form (5 × 25 mm). The column is thoroughly washed with water and the sample eluted with 200 mM ammonium hydroxide, neutralized with acetic acid, and freeze-dried. The product may contain residual 2-aminopyridine (particularly if the oligosaccharide being derivatized contains only one or two saccharide residues) and can be further purified if necessary.25

3. The 2-aminopyridine derivatives of monosaccharides11 and neutral oligosaccharides14 (such as those prepared from ovalbumin by hydrazinolysis) can be analyzed in 200 mM potassium borate buffer at pH 10.5.11 Electrophoresis is performed in a 65- to 95-cm fused silica capillary at 15–20 kV. Injection is at the anodic end and detection is at the cathodic end with ultraviolet at 240 nm or by fluorescence (λ_ex = 316 nm, λ_em = 395 nm).

4. Maltooligosaccharides derivatives (tetrasaccharides through heptasaccharides) can be analyzed in 0.1 M sodium phosphate containing 50 mM tetrabutylammonium bromide at pH 5.0.12 Electrophoresis is performed in a 50-cm fused silica capillary at 15 kV. Injection and detection is as described in step 3.

Conjugation with Charged Tag

High-resolution separation and high-sensitivity fluorescence detection of sugars can be achieved by using saccharide conjugation to a fluorescent tag containing a fixed charge (Fig. 2b).

1. The monopotassium salt of 7-amino-1,3-naphthalenedisulfonic acid [amido-G acid (AGA)] is used after recrystallization from deionized water. Oligosaccharide (3.5 μmol) is dissolved in 750 μl of AGA solution [50% (w/v) in water adjusted with sodium hydroxide to pH 6.2]. After heating at 80° for 60 min, sodium cyanoborohydride (16 μmol) is added. The mixture is then heated for 24 hr at 70°. After the reaction is complete the products are fractionated on a BioGel P-4 (Bio-Rad, Richmond, CA) or Sephadex G-25 column (2.5 × 50 cm) eluted with distilled water to remove salt and excess fluorescent tag. The sugar conjugate (Fig. 2b) is recovered in the void or included volume of the column and is detected either by using a hand-held ultraviolet light (long wavelength) or in a spectrometer by absorbance at 314 nm or by fluorescence (λex = 314 nm and λem = 452 nm).

2. Oligosaccharide–AGA conjugates can be purified to homogeneity, if desired, by gradient preparative polyacrylamide gel electrophoresis, using a linear gradient from 12 to 22% (w/v) acrylamide that contains 0.5 to 2% (w/v) N,N'-bisacrylamide. Electrophoresis is done on a gel (32 × 16 × 0.75 cm) for 18 hr at 400 V (constant voltage) with cooling. Following electrotransfer onto positively charged nylon membranes, oligosaccharide–AGA conjugates are recovered by washing the membranes with 2 M sodium chloride. The salt is removed, using a BioGel P-2 column eluted with distilled water, and the sample is freeze-dried.

3. Electrophoresis is performed with a CE system in a fused silica capillary tube (68 cm long) by gravity injection (10 nl) at 20 kV, using 10 mM sodium borate and 50 mM boric acid, having a pH of 8.8.

4. Fluorescence detection requires the installation of a long-pass optical emission filter in the cell. To optimize detection, an emission filter of 420 nm is used. The excitation wavelength is varied at 50-nm intervals to obtain the optimum excitation wavelength. With the optimum excitation wavelength various emission filters are tested to determine which gives the greatest sensitivity. Optimum detection sensitivity by fluorescence [on a Dionex (Sunnyvale, CA) capillary electrophoresis system] is obtained at λex = 250 nm and λem = 420 nm.

Comments on Method. Reductive amination is a useful method for labeling sugars with probes to permit their detection and facilitates their separation. Amido-G acid contains two fixed negative charges used to drive a separation by electrophoresis. The greater the number of sugar residues in a particular oligosaccharide, the more vigorous are the conditions (i.e., longer reaction times, higher temperatures) required to ensure

complete derivatization.\textsuperscript{15} For oligosaccharides having a terminal hexose, a yield of 90\% is possible, whereas for N-acetylhexosamine reducing ends recovered yields of 60–80\% are obtained. Neutral pH is used when derivatizing sugars containing acid-labile functionalities such as sialic acids.\textsuperscript{15} Detailed studies may be required to optimize yield for each type of oligosaccharide being labeled.

Oligosaccharide-fluorescent conjugates prepared are typically contaminated with excess fluorescent tag (AGA) required to drive the reaction to completion. It is often possible to remove this excess reagent by gel-filtration chromatography. Often, however, the oligosaccharide being derivatized is not 100\% pure. These impurities can lead to the formation of additional minor products during reductive amination. Preparative gradient polyacrylamide gel electrophoresis can be used to resolve, identify, and recover the desired product(s) in the crude reaction mixture.

Fluorescence detection of AGA–oligosaccharide conjugates is sensitive. A linear calibration curve constructed for the AGA derivative of chitotriose gave a linear response from 42 fmol to 1 pmol.

**Capillary Zone Electrophoresis for Assaying Glycosyltransferases**

Glycosyltransferases are an important group of enzymes that transfer sugar residues from specific activated sugar nucleotide donors to suitable oligosaccharide acceptors. A method that relies on capillary electrophoresis for the assay of glycosyltransferases has been reported that uses a sugar–AGA conjugate as acceptor molecules. In glycosyltransferase assays both sugar–conjugate acceptor and sugar–conjugate product are rapidly quantified by CZE.

1. $\beta$-D-Gal-(1 $\rightarrow$ 4)-$\beta$-D-GlcNAc-(1 $\rightarrow$ 6)-$\beta$-D-Gal is conjugated to AGA by reductive amination in the presence of sodium cyanoborohydride (see above). The products are desalted on a 2.5 $\times$ 50 cm BioGel P-2 column and purified by preparative PAGE.

2. The AGA–acceptor (10 $\mu$g) is incubated at 37\° for 26 hr with $\alpha$(1 $\rightarrow$ 3/4)-fucosyltransferase (13 $\mu$U) and GDPfucose (420 $\mu$mol) in 150 $\mu$l of 16 mM sodium cacodylate buffer at pH 6.8, containing 3 mM manganese chloride. The mixture is freeze-dried at the end of the reaction.

3. The product mixture (0.5 mg/ml) is reconstituted in distilled, deionized water. Analysis is performed on a CE system with fluorescence detection (as described above).

**Comments on Method.** Capillary zone electrophoresis analysis of the purified oligosaccharide–AGA acceptor, using fluorescent detection, showed a single major peak. The $\alpha$(1 $\rightarrow$ 3/4)-fucosyltransferase reaction
showed an additional peak that migrates faster than the acceptor, corresponding to product. These results are consistent with CZE studies that show that larger sugar–AGA conjugates migrate faster than smaller sugar–AGA conjugates. The presence of α-linked fucose at the nonreducing end of the product is confirmed by conversion of product to acceptor on treatment with α-L-fucosidase.

This approach may represent a general method to assay various glycosyltransferase activities, including N-acetylgalactosaminyltransferases, fucosyltransferases, sialyltransferases, and mannosyltransferases, provided proper sugar–AGA acceptors are prepared for each. The sensitivity of this CZE analysis is 80 fmol. Time-course studies that measure transfer of the activated sugar to oligosaccharide–AGA acceptor are also possible.

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