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An Electrophoresis-Based Assay for Glycosyltransferase Activity

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Polyacrylamide gel electrophoresis (PAGE) and capillary zone electrophoresis (CZE) were used to measure the activity of glycosyltransferases. Acceptor molecules were prepared by reductive amination of the monopotassium 7-amino-1,3-naphthalenedisulfonic acid (AGA) Schiff base with sugars. The resulting sugar conjugates were purified by gradient PAGE and recovered using semidry electrotransfer into a positively charged nylon membrane. The β(1→4)galactosyltransferase was shown, by PAGE analysis, to transfer a β-galactosyl residue to the AGA conjugate of β-D-GlcNAc-(1→4)-β-D-GlcNAc-(1→4)-D-GlcNAc (compound 4). Similarly, α(1→2)fucosyltransferase isolated from porcine submaxillary glands was shown to transfer fucose from GDP–fucose to the AGA conjugate of β-D-Gal-(1→4)-β-D-GlcNAc-(1→6)-D-Gal (compound 5). This conjugate (compound 5) was also an acceptor for the α(1→3/4)fucosyltransferase partially purified from human milk. The latter reaction was followed by both gradient PAGE and CZE, having sensitivities of 200 pmol and 80 fmol, respectively. © 1992 Academic Press, Inc.

The carbohydrate components of glycoproteins and glycolipids play many important biological roles (1). They are biosynthesized through the sequential action of various glycosyltransferases and glycosidases (2). Glycosyltransferases transfer sugar residues from specific activated sugar nucleotide donors to suitable oligosaccharide acceptors. The action of these important enzymes is usually measured by detecting the radiolabeled sugar being transferred from the sugar nucleotide to the oligosaccharide acceptor (3–7). The use of radiolabeled sugar nucleotides for assaying glycosyltransferases has several disadvantages. These assays can be time-consuming, requiring procedures to separate the radiolabeled products from the reaction mixture. In addition, the use of radiolabels can pose biological hazards and disposal problems.

Morita *et al.* (8) recently developed a nonradiochemical method of assaying glycosyltransferases using a fluorescently labeled (pyridylaminated) sugar as an acceptor molecule. Separation and analysis were performed using reversed-phase high-performance liquid chromatography (HPLC).[2] Methods that rely on gel or capillary electrophoresis for the assay of glycosyltransferases have not been reported. This is probably due to the absence of a fixed charge on most sugars that would be required to drive these separations.

In this study, we describe an electrophoresis-based assay of galactosyltransferase (EC 2.4.1.22) and fucosyltransferase (EC 2.4.1.65 and 2.4.1.69) activities using sugar–fluorescent conjugates (7-amino-1,3-naphthalenedisulfonic acid) as acceptor molecules. This method introduces two fixed charges, in the form of sulfonate, onto a neutral sugar by the use of reductive amination (9). The sugar conjugates are purified by preparative gradient polyacrylamide gel electrophoresis (PAGE) followed by semidry electrotransfer (10). The fluorescent, charged tags, described in this paper, facilitate the analysis of neutral oligosaccharides by both PAGE and capillary zone electrophoresis (CZE). The glycosyltransferase assays using these sugar conjugates can be

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[2] Abbreviations used: UDP-Gal, uridine 5′-diphosphogalactose; GDP-Fuc, guanosine 5′-diphosphofucose; PAGE, polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; SAX-HPLC, strong anion-exchange high-performance liquid chromatography; AGA, monopotassium 7-amino-1,3-naphthalenedisulfonic acid; FAB, fast atom bombardment; CZE, capillary zone electrophoresis; TSP, 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt; NMR, nuclear magnetic resonance; RP, reversed-phase.

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performed qualitatively on gradient PAGE or quantitatively by CZE (11).

MATERIALS AND METHODS

Materials

Chitotriose [β-D-GlcNAc-(1→4)-β-D-GlcNAc-(1→4)-D-GlcNAc] **1**, β-D-Gal-(1→4)-β-D-GlcNAc-(1→6)-D-Gal **2**, β-galactosidases (EC 3.2.1.23, from jack beans, *Escherichia coli,* and *Aspergillus niger*), α-L-fucosidase (EC 3.2.1.51, from bovine kidney), β-galactosyltransferase (EC 2.4.1.38), sodium cyanoborohydride, UDP-Galactose, sodium cacodylate, and Triton X-100 were from Sigma Chemical (St. Louis, MO). GDP-fucose was synthesized as previously described (12). CM-Sephadex C-50 and SP-Sephadex were from Pharmacia (Piscataway, NJ). The β-galactosidases from *Diplococcus pneumoniae* and bovine testes were obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN). Monopotassium 7-amino-1,3-naphthalenedisulfonic acid (Amido-G-Acid, AGA, **3**), ^2H_2O (99.96 at. % D) and TSP (99 at. % D) were from Aldrich (Milwaukee, WI). Bio-Gel P2 was from Bio-Rad (Richmond, CA). Reagents used for PAGE were from Fisher Chemical Co. (Fair Lawn, NJ). Biotrace RP nylon (positively charged nylon membrane) and nitrocellulose membranes were from Gelman Science Inc. (Ann Arbor, MI). The 3-mm filter paper was from Whatman (Hillsboro, OR). The 32 × 16-cm vertical slab gel unit (SE 620), and the TE70 electrophoresis transfer unit were obtained from Hoefer Scientific Instruments (San Francisco, CA). The electrophoresis power unit Model 1420B was from Bio-Rad. The transilluminator (TR-365A), uv-transmitting filter protector (UVT-150), and diffusing screen were purchased from Spectronics Co. (Westbury, NY). Strong anion-exchange HPLC used Shimadzu Bio Liquid LC-7A pumps (Kyoto, Japan) and a 2141 ultraviolet detector from Pharmacia LKB Biotechnology, Inc. SAX-HPLC used a Spherisorb (5 μm particle size, 4.6 mm × 25 cm) column of dimensions from Phase Separtions (Norwalk, CT). Capillary zone electrophoresis used a Dionex (Sunnyvale, CA) capillary electrophoresis system with uncoated silica capillary (0.75 μm × 65 cm).

Methods

Preparation of fucosyltransferases. Partially purified α(1→3/4)fucosyltransferase (EC 2.4.1.65) was isolated from human milk and assayed by a modification (13) of reported procedures (14,15). Isolation steps included precipitation with 65% ammonium sulfate and successive chromatography on CM-Sephadex C-50 and GDP-hexanolamine–agarose. GDP-hexanolamine was synthesized and coupled to agarose as previously described (15). The α(1→3/4)fucosyltransferase preparation was devoid of α(1→2)fucosyltransferase activity when assayed with β-Gal-*O*-$(CH_2)_8$COOMe as a substrate. The β(1→2)fucosyltransferase (EC 2.4.1.69) was isolated from porcine submaxillary glands (16). Isolation steps included extraction with Triton X-100 and chromatography on SP-Sephadex and GDP-hexanolamine–agarose.

Preparation of fluorescently labeled sugars as glycosyltransferase acceptors. Sugar–AGA conjugates were prepared by reductive amination in the presence of sodium cyanoborohydride as previously described (9). The monopotassium salt of AGA was used after recrystallization from double-distilled deionized water. Sugar (3.5 μmol) was dissolved in 750 μl of AGA **3** solution [50% (w/v) in water adjusted with sodium hydroxide to pH 6.2]. Gradual heating to 80°C over a period of 2 h was followed by addition of sodium cyanoborohydride (16 μmol) as a solid (the pH changed by less than 0.1). The mixture was heated for 12 h at 70°C in an incubator shaker after which additional sodium cyanoborohydride (16 μmol) was added and incubated for 12 more hours. After the reaction was complete, the products were desalted on a 2.5 × 50-cm Bio-Gel P2 column eluted with distilled water. The samples were freeze-dried and reconstituted in 100 μl of distilled water before loading on the preparative gel.

Purification of sugar–AGA conjugates. Sugar–AGA conjugates were purified by preparative PAGE using a linear gradient of 12 to 22% (w/v) acrylamide that contained 0.5–2% (w/v) *N,N'*-bisacrylamide (9). Electrophoresis was performed for 7 h at 400 V (constant voltage). Sugar–fluorescent conjugate was transferred from gel to positively charged nylon membrane by semidry electrotransfer (9,10) and the sugar–fluorescent conjugates were recovered from the membrane by washing with 2 M sodium chloride. Sodium chloride was removed using a desalting Bio-Gel P2 column eluted with distilled water.

Incubation procedure for galactosyltransferase. To 1.25 ml of premixed incubation buffer solution (65 mM NaCl, 2.7 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), pH 6.0, 10 mM $MnCl_2$, 500 μg chitotriose–AGA conjugate (compound **4**), 5 mg UDP-Gal, and 5 U β-galactosyltransferase were added (8), and the mixture was incubated at 37°C for 10 h. The formation of product **6** was demonstrated by loading a small portion of incubation mixture directly on an analytical gradient polyacrylamide gel without further purification. A large scale galactosyltransferase reaction was also performed in which a fivefold increase in reactants and enzyme was used.

Incubation procedure for α(1→2)fucosyltransferase. Incubation mixtures were carried out in 1.5-ml microcentrifuge tubes containing in 55 μl of 0.59 mM GDP-Fuc (14), 10 μg acceptor **5**, 40 μU α(1→2) fucosyltransferase in 18 mM sodium cacodylate buffer, pH 6.5, with 4 mM $MnCl_2$. The reaction was initiated by the addition

of nucleotide donor; the samples were mixed briefly using a vortex mixer, centrifuged, and then incubated at 37°C for 24 h. At the end of the incubation period, the samples were dried under reduced pressure on a Savant Speed-Vac centrifuge. To confirm the presence of fucose at the nonreducing end, the product **7** was treated with α-fucosidase (9). A parallel incubation that lacked GDP-Fuc served as a control.

Incubation procedure for α(1→3/4)fucosyltransferase. The incubations with α(1→3/4)fucosyltransferase were carried out in 1.5-ml microcentrifuge tubes containing in 155 μl of 0.42 mM GDP-Fuc, 10 μg acceptor **5**, 13 μU α(1→3/4)fucosyltransferase in 16 mM sodium cacodylate buffer, pH 6.8, with 3 mM $MnCl_2$. These samples were incubated at 37°C for 26 h and then dried under reduced pressure. A parallel incubation that lacked GDP-Fuc served as a control. To confirm the presence of fucose at the nonreducing end, the product **8** was digested with α-fucosidase (9).

Purification and isolation of enzymatic products. Reaction mixture solution was desalted by using Bio-Gel P2 low pressure column (2.5 × 75 cm). The sample was freeze-dried and reconstituted in 1 ml of double-distilled water. The sample was then purified by gradient PAGE using the procedures previously described for the purification of sugar–AGA conjugates.

Identification of reaction products by β-galactosidase digestion. To identify the sugar at the nonreducing end and its linkage position, the purified product **6** (approximately 1 μg) was digested overnight at 37°C with 75 mU β-D-galactosidase from *D. pneumoniae* (EC 3.2.1.23) or with 10 mU β-galactosidase from bovine testes (EC 3.2.1.23) in 100 μl of buffer comprised of Tris–HCl (20 mmol/liter), KCl (100 mmol/liter), and EDTA (1 mmol/liter), at pH 7.5. The digestion mixtures were analyzed using analytical gradient PAGE after a digestion time of 12 h. The partially purified product **6** recovered from the large scale galactosyltransferase reaction using semidry electrotransfer was further purified by preparative SAX-HPLC, using a 60 min linear gradient (0 to 1 M) of sodium chloride (9). Absorbance and the conjugate's fluorescence were used to monitor the elution pattern. The product purified by SAX-HPLC was desalted on a Bio-gel P2 column and freeze-dried. This was again subjected to β-galactosidase digestion as well as analysis by NMR spectroscopy.

Photography. The pictures of gel were taken in a dark room on the transilluminator (at 365 nm), using a diffusing screen between the lamp and the gel. ATMX-100 black and white 35-mm film (Kodak), a uv filter, and an aperture setting of f2.8, with automatic shutter speed, were used.

Analyses by proton NMR spectroscopy and FAB mass spectrometry. ^1H NMR spectroscopy was performed using a Bruker AMX-600, operating at ^1H frequency of 600 MHz and 25°C. Purified acceptor **4** and product **5** (~250 μg) were each dissolved and freeze-dried from deuterium oxide (99.9 at. % D) three times. The samples were then prepared in deuterium oxide (99.96%) containing TSP as the internal standard. Negative-ion FAB mass spectra were obtained on a VG ZAB-HF spectrometer using triethanolamine as the matrix (17). Sample solutions were prepared by dissolving the freeze-dried sugar–AGA conjugate **6** in water at a concentration of 10–20 μg/μl. A mixture of sample solution (1 μl) and triethanolamine (1 μl) was placed on the FAB probe tip for analysis.

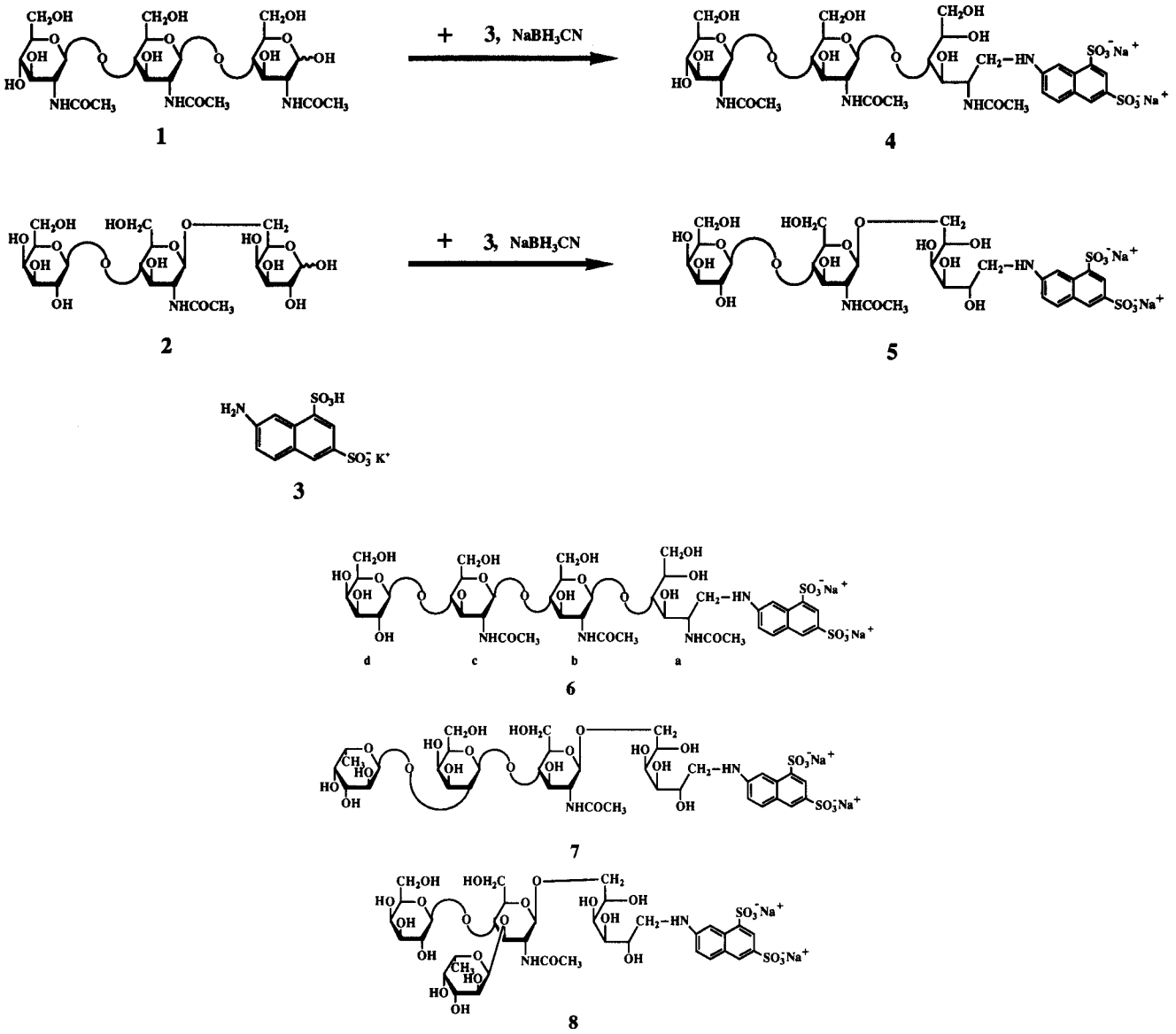
Capillary zone electrophoresis analyses. The samples (10 μg/20 μl) were prepared in double-distilled deionized water. The capillary tube was activated by washing with 0.1 M phosphoric acid, 0.5 M sodium hydroxide, and deionized water. The column was eluted with sodium borate buffer (10 mM sodium borate and 50 mM boric acid, pH 8.8). Electrophoresis was performed at 20 kV after gravity injection of 1 or 4 nl with fluorescence detection (excitation at 250 nm, emission at 420 nm).

RESULTS AND DISCUSSION

The preparation of sugar–AGA conjugates **4** and **5**, the transferase acceptors, using reductive amination is shown in Scheme 1. Oligosaccharide **2** readily conjugates to AGA in high yields (>80%) and chitotriose (**1**) in slightly lower yields (~70%). Recovered yields, based on oligosaccharide starting material, compared well with yields estimated from band intensity by using gradient PAGE.

The applicability of conjugate **4** as a bovine milk galactosyltransferase acceptor was first examined using UDP-Gal as a sugar donor. The reaction mixture was analyzed directly by the gradient PAGE method used in the purification steps. The galactosyltransferase reaction product **6** was a tetrasaccharide as judged by migration distance on gradient PAGE compared to previously characterized standards (9) (Fig. 1, lane B). After treating with β-galactosidase (bovine testes), the galactosyltransferase reaction product was completely converted into a compound having the same electrophoretic mobility as the chitotriose–AGA conjugate acceptor **4** (Fig. 1, lanes B and C). This experiment demonstrated that the nonreducing end of the fluorescent product formed in the galactosyltransferase reaction was β-linked galactose.

Negative-ion FAB-MS was performed to determine the molecular weight of the β-galactosyltransferase reaction product **6.** Ions were observed at *m/z* 1119, 1097, and 1075, corresponding to molecular ions $[M-H]^-$, $[M-Na]^-$, and $[M-2Na+H]^-$, respectively. A fragment ion was observed at *m/z* 708 that corresponded to the loss of two sugars (one galactose and one *N*-acetylglucosamine) residue from the nonreducing end. The ion



SCHEME 1. Synthesis of transferase acceptors.

corresponding to the loss of only the terminal, transferred galactosyl residue was not observed in the FAB mass spectrum.

The galactosyltransferase reaction product **6** was also examined by high field ^{1}H NMR spectroscopy. To characterize the reaction product, a preparative scale (fivefold higher concentration than prepared for analytical PAGE analysis) reaction was performed and the product **6** was purified by gradient PAGE and SAX-HPLC. Signal assignment was made on the basis of published data (18). The assignments for the oligosaccharide–AGA acceptor **4** were as follows (in ppm): 1.91 (-CO$\underline{CH_3}$), 1.96 (-CO$\underline{CH_3}$), 2.07 (-CO$\underline{CH_3}$), 7.22 (dd, H-6, J = 8.9 and 2.2 Hz), 7.67 (H-8, J = 2.2 Hz), 7.94 (d, H-5, J = 9.0 Hz), 8.31 and 8.33 (H-2 and H-4, signals interchangeable). Two anomeric proton signals observed at 4.51 ppm (d, J = 7.8 Hz) and 4.60 ppm (d, J = 8.4 Hz) could be assigned to H-1c and H-1b of internal β-D-GlcNAc residues. The signals for the galactosyltransferase product **6** were assigned as follows (in ppm): 1.91 (-CO$\underline{CH_3}$), 1.96 (-CO$\underline{CH_3}$), 2.07 (-CO$\underline{CH_3}$), 7.22 (d, H-6, J = 8.7 Hz), 7.67 (H-8, not resolved), 7.94 (d, H-5, J = 9.0 Hz), 8.31 and 8.33 (H-2 and H-4, signals interchangeable), 4.46 (d, H-1c of β-D-GlcNAc, J = 7.9 Hz), 4.60 (d, H-1b of β-D-GlcNAc, J = 8.5 Hz), 4.52 (d, H-1d of β-D-Gal, J = 8.1 Hz). The anomeric proton signal of terminal N-acetylglucosamine (H-1c) was shifted from 4.51 to 4.46 ppm due to the introduction of galactose through a β-linkage to the terminal N-acetylglucosamine residue. The shift of the anomeric proton signal, derived from the internal N-acetylglucosamine (H-1b), remained at 4.60 ppm (as in chitotriose–AGA conjugate

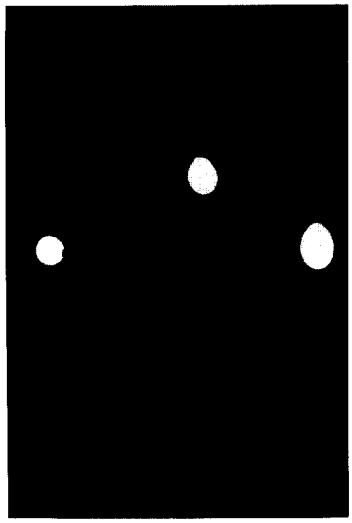


FIG. 1. Gradient PAGE analysis of substrate **4** and β-galactosyltransferase product **6**. Lane A, 1 μg substrate **4**; lane B, 1 μg of β-galactosyltransferase product **6**; lane C, 1 μg of β-galactosyltransferase product **6** treated with 10 mU of β-galactosidase (bovine testes).

4). In conclusion, the ^{1}H NMR spectrum was consistent with only a single reaction product of the expected structure.

To better define the identity of the transferase product, the reaction mixture was subjected to hydrolyses using both specific and nonspecific β-galactosidases. β-Galactosidase from bovine testes is a nonspecific hydrolyase while the one from *D. pneumoniae* specifically cleaves only β-D-Gal-(1→4)-D-GlcNAc linkage and not β-D-Gal-(1→3)-D-GlcNAc or β-D-Gal-(1→6)-D-GlcNAc linkages. When the partially purified reaction mixture was treated with a nonspecific β-galactosidase complete conversion of **6** to **4** was observed, as expected, demonstrating that the β-galactosyl residue was transferred to the nonreducing end of oligosaccharde–AGA acceptor **4**. However, when the partially purified β-galactosyltransferase reaction product **6** was treated at 37°C for 12 h with specific β-galactosidase from *D. pneumoniae* and the reaction was analyzed by gradient PAGE, only 60–70% of sample **6** was converted to chitotriose–AGA **4** (not shown). This incomplete hydrolysis could result from the reduced susceptibility of sugar–AGA conjugate **6**, due to a contaminant present in the product mixture that acted as an inhibitor of this β-galactosidase, or the presence of a minor product resistant to this specific β-galactosidase (i.e., one containing a 1→3 or 1→6 linkage). When the product **6** was fractionated using SAX-HPLC, only a single peak was observed which showed fluorescence. When this purified product was subjected to specific β-D-galactosidase from *D. pneumoniae* under identical experimental conditions, complete conversion of **6** to **4** was observed. This suggests a contaminant in the semipurified product mixture obtained through electrotransfer, which had inhibited this β-galactosidase, was removed by SAX-HPLC. These data together with the NMR spectrum of this product show that the transfer is highly specific, giving only a 1→4 linked β-galactosyl residue at the nonreducing terminus.

Fucosyltransferase activity was also studied using conjugate **5** as an acceptor molecule. GDP-Fuc served as a sugar donor and the reaction mixture was analyzed directly by gradient PAGE. In the first reaction, GDP-Fuc and acceptor **5** were incubated with α(1→2)fucosyltransferase. A product with a greater molecular size than that of acceptor **5** was observed on gradient PAGE analysis (Fig. 2, lanes A and B). In addition to the formation of this new band, the intensity of the band corresponding to acceptor **5** also decreased. Treatment the reaction mixture containing with 10 mU of α-L-fucosidase (bovine kidney) gave a single band corresponding to **5** (Fig. 2, lane C). Thus, the nonreducing sugar in the fluorescently tagged product **7** was α-linked fucose. In a second transferase reaction, GDP-Fuc and acceptor **5** were incubated with α(1→3/4)fucosyltransferase. A product, **8**, with a greater size than the acceptor **5** (Fig. 3, lane B) was again observed on gradient PAGE analysis (Fig. 3, lane A). This time only a very small amount of acceptor **5** remained. The single new band resulting from the transferase reaction indicates the incorporation of α(1→3)-linked fucose. The shift observed for the band formed in the transferase reaction is, furthermore, consistent with the addition of a single fucose residue. The preparation of standards will be required to establish the precise structure of this product. Again treatment of the reaction mixture with fucosidase (bovine

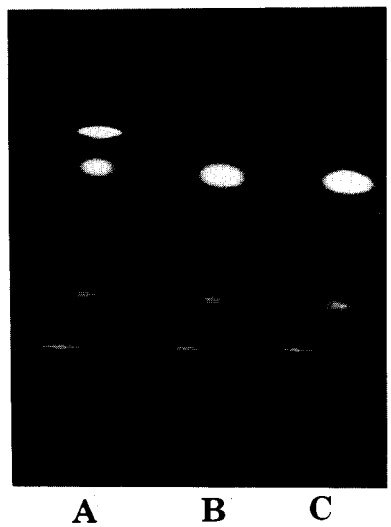


FIG. 2. Gradient PAGE analysis of α(1→2)fucosyltransferase reaction mixture. Lane A, 1 μg of transferase product **7** (containing residual **5**); lane B, 1 μg of transferase acceptor **5**; lane C, 1 μg of transferase product treated with 10 mU of bovine kidney fucosidase.

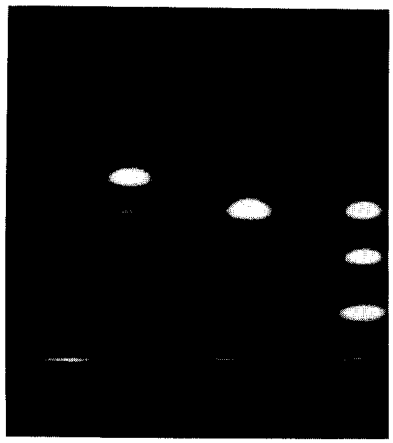


FIG. 3. Gradient PAGE analysis of $\alpha(1\rightarrow3/4)$fucosyltransferase reaction mixture. Lane A, 4 μg of product **7**; lane B, 4 μg of acceptor **5** (control); lane C, 4 μg of product **7** treated with 100 mU of bovine kidney fucosidase. The additional bands observed corresponding in size to monosaccharide- and disaccharide-AGA conjugates may result from glycosidase contamination of this fucosidase. This was not a problem in Fig. 2 where only 10 mU of bovine kidney fucosidase was used.

kidney) confirmed the presence of an α-linked fucosyl residue at the nonreducing end (Fig. 3, lane C). The use of excess fucosidase (100 mU) resulted in two additional bands. These more mobile bands correspond in size to monosaccharide–AGA and disaccharide–AGA conjugates and suggest the presence of contaminating glycosidases in this fucosidase preparation.

The interpretation of the fucosyltransferase experiments relies on changes in the product mobility on gradient PAGE. Despite its relative ease of use and widespread availability, gel electrophoresis has certain limitations, particularly in sample quantitation and ease of automation. Thus, we examined CZE in an effort to overcome these limitations. CZE has been shown to provide a convenient method for the quantitative analysis of mixtures of oligosaccharides (11). CZE analysis, using fluorescence detection, shows a major peak for the purified oligosaccharide–AGA acceptor **5** (Fig. 4A). The $\alpha(1\rightarrow3/4)$fucosyltransferase reaction showed a single additional peak corresponding to product **8** that migrated faster than the acceptor **5** (Fig. 4B). This was consistent with earlier CZE studies (11) that show that larger sugar–AGA conjugates migrate faster than smaller sugar–AGA conjugates. Because of the variation observed in retention time, peak assignment was confirmed by a comigration experiment. Coinjection of transferase acceptor and product identified the newly formed peak as **8** and confirmed that it did indeed migrate faster than the oligosaccharide–AGA acceptor **5** (Fig. 4C). CZE analysis gave results consistent with the PAGE analysis, demonstrating that product **8** was at a higher concentration than the acceptor **5** (Fig. 4B). Again the presence of a single product with α-linked fucose was confirmed by the complete conversion of **8** to **5** on digestion of the reaction mixture with α-fucosidase (Fig. 4D). The lack of appropriate standards prevented us from determining whether CZE could separate the various fucosylated products. If this high resolution method can be used to achieve such separations then

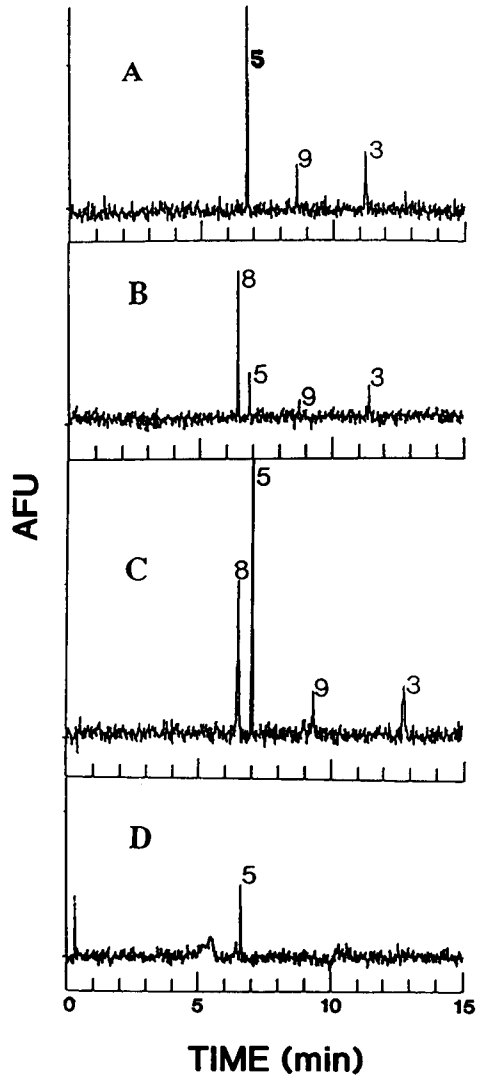


FIG. 4. CZE analysis of $\alpha(1\rightarrow3/4)$fucosyltransferase reaction mixture. A, 0.1 ng of substrate **5**; B, 0.1 ng of product **8**; C, 0.1 ng of a 1:1 (v/v) mixture of substrate **5** and product **8**; D, 0.09 ng of product treated with bovine kidney fucosidase (0.5 mU fucosidase/μg product). In A, B, and C a small quantity of Gal–AGA **9** and AGA **3** contaminating the acceptor preparation was also observed. Gal–AGA conjugate **9** is not present in the purified acceptor **5**. It appears instead during incubation of the pure acceptor **5** with fucosyltransferase in the absence of GDP-Fuc (control experiment) and may be caused by a minor amount of contaminating glycosidase activity in the transferase preparation.

the transferase content of crude enzyme preparations or tissue extracts could be examined.

In conclusion, we have demonstrated a useful new assay method for galactosyltransferase and fucosyltransferase activity. This approach may represent a general method to assay various glycosyltransferase activities including *N*-acetylglucosaminyltransferases, fucosyltransferases, sialyltransferases, and mannosyltransferases, providing proper sugar–AGA acceptors can be prepared for each. The sensitivity of PAGE analysis is 200 pmol/band and that of CZE analysis is 80 fmol. These methods offer high sensitivity alternative to the use of radiolabels for glycosyltransferase assays.

CZE analysis might also permit a time-course study of transfer of the activated sugar to oligosaccharide–AGA acceptor in which both the disappearance of acceptor and the appearance of product also could be monitored. Such a time-course study, using CZE, has been reported on the breakdown of chitooligosaccharides with chitinase (11). Currently, radiolabeled sugar nucleotides are primarily used for the assaying of glycosyltransferase activity. These methods often require tedious separation methods including paper electrophoresis (19), thin-layer chromatography (20), high-performance thin-layer chromatography (21), and HPLC (22). The use of a fluorescent tag may also represent a safer alternative to the use of a radiolabeled sugar nucleotides.

This study also suggests another potential use of this method. Biologically active, fluorescently tagged compounds might be synthesized through the action of glycosyltransferases in a stereospecific and regiospecific manner. Using oligosaccharide–AGA acceptors, longer oligosaccharide chains might be elaborated. Each intermediate, in this enzymatic synthesis, and the final product could be easily isolated and purified using preparative gradient PAGE. Additional studies on the general utility of these reactions need to be studied before this approach will have value as a synthetic method.

ACKNOWLEDGMENTS

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