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The analysis of oligosaccharides derived from different sources by fluorophore-assisted carbohydrate electrophoresis

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Abstract

Fluorophore-assisted carbohydrate electrophoresis (FACE) is a straightforward, sensitive method for determining the presence and relative abundance of individual (oligo)saccharide in a(n) (oligo)saccharide mixture. The single terminal aldehydes of oligoglucoside residues released by acid hydrolysis of β-1, 3-D-glucan from yeast were tagged with the charged fluorophore 8-aminonaphthalene-1,3,6-trisulfonate (ANTS), and separated with high resolution on the basis of size by polyacrylamide gel electrophoresis. ANTS fluorescence labeling was not biased by oligoglucoside length; therefore, band fluorescence intensity was directly related to the relative abundance of individual oligoglucoside moieties in heterogeneous sample. Therefore, FACE represents an accessible, sensitive, and quantitative analytical tool enabling the analysis of (oligo)saccharides derived from different sources.

Keywords: Fluorophore-assisted carbohydrate electrophoresis; Analysis; Different (oligo)saccharides

1. Introduction

The structural specifics and subtleties of the (oligo)saccharides may be ascertained by the use of sophisticated analytical tools, such as multidimensional nuclear magnetic resonance (NMR). Unfortunately, the difficulty of data interpretation and the scarcity of (oligo)saccharide NMR expertise limit rapid and widespread use of this analytical approach. A simple method for determining the presence and relative abundance of (oligo)saccharides from different sources will contribute to our understanding of the biological functions of these (oligo)saccharides.

Fluorophore-assisted carbohydrate electrophoresis (FACE) is a high-resolution polyacrylamide gel electrophoretic procedure that separates oligosaccharides by size (Jackson, 1990). Individual carbohydrate moieties are tagged at the terminal aldehyde with the highly charged fluorophore 8-aminonaphthalene-1,3,6-trisulfonate (ANTS), which imparts a uniformly strong negative charge to each oligosaccharide or monomeric reducing sugar and enables using polyacrylamide gel electrophoresis (PAGE) separation. The relative abundance of each saccharide residue present in the starting mixture is represented by the fluorescence intensity of the resulting band on the gel (Jackson, 1990; Hu & Vallee, 1994; Stack & Sullivan, 1992).

In this report, we describe the application of FACE for determining the relative abundances and the relative migration indices (RMI) of oligosaccharides released by acid hydrolysis of wheat starch and dextran, respectively. We demonstrate that the products obtained by acid hydrolysis, are amenable to FACE analysis.

2. Materials and methods

2.1. Materials

Maltose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, *N*,*N*′-methylenebisacrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), ammonium persulfate, ANTS and sodium cyanoborohydride were purchased from Sigma–Aldrich Chemical Company. SigmaGel gel

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analysis software was purchased from SPSS Science Inc., Chicago, IL.

2.2. Partial acid hydrolysis of wheat starch and dextran

To prepare hydrolysates, 50–100 mg of wheat starch or dextran were suspended at a concentration of 0.5% hydrochloric acid, heated at 60 °C for 110 min in 25-mL reactiflasks. Cooled, and centrifuged at $160 \times g$ for 10 min at 25 °C. Supernatant fractions from 10 reactiflasks were pooled and the residual hydrochloric acid was removed by rotoevaporation at 35–40 °C in a silanized round bottom flask. The sample was lyophilized.

2.3. ANTS labeling of oligosaccharides

The dried oligosaccharides sample was suspended in 5.0 mL of 0.2 mol/L ANTS in acetic acid–water (3:17, v/v) and freshly made 1.0 mol/L sodium cyanoborohydride in dimethyl sulfoxide and incubated at 37 °C for a period of time. The sample was dried under nitrogen at 45 °C, suspended in 50 mL of loading buffer (62.5 mmol/L Tris–HCl, pH 6.8, containing 20% glycerol), and stored at −70 °C.

2.4. Electrophoresis of ANTS-labeled oligosaccharides

The resolving gel was 32% acrylamide–2.4% bisacrylamide in a 140- by 160- by 0.75-mm glass cassette. For every 35 mL of resolving gel, 150 μL of 10% ammonium persulfate and 15 μL of TEMED were added. The stacking gel was 8% acrylamide −0.6% bisacrylamide containing 50 μL of 10% ammonium persulfate and 5 μL of and TEMED, respectively, for every 6 mL of stacking gel. The running buffer and the gel buffer were 0.025 mol/L Tris base–0.192 mol/L glycine (pH 8.4) and 0.42 mol/L Tris base (pH 8.5), respectively. Electrophoresis was run at a constant current of 15 mA for 6 h in a cooled buffer system.

2.5. Visualization, photography, and image analysis

For visualization of the ANTS-labeled oligosaccharides, the gel was removed from the glass cassette and placed onto the surface of a light box with ultraviolet (UV) illumination (300 nm). The gels were photographed through a No. 12 Kodak Wratten gelatin filter with Polaroid type 57 film, at a film speed of ISO 3000/36°, at f 11 and an exposure time of 3–10 s. The photographs were scanned by using a Hewlett-Packard ScanJet 6200C at a resolution of 300 dpi and the images were inverted (inverse pixels) using Adobe Photoshop 4.0. The tagged-image format file (TIFF)-based images were analyzed using SigmaGel gel analysis software. The oligosaccharide concentration in the individual bands, defined as regions exhibiting intensities of >10% of background, was calculated based on band fluorescence intensity (pixel number). RMI of the oligosaccharides (x) were calculated based on the migration of each oligosaccharide relative to a mixture of malto-oligosaccharides of known structure derived from the acid-hydrolyzed dextran, by the equation $\mathrm{RMI}_x = [(d_x - d_n)/(d_{n+1} - d_n)] + n$, where d is the distance migrated and n is the number of glucose residues in the oligosaccharide.

3. Results and discussion

3.1. FACE labeling chemistry and reaction kinetics

FACE involves labeling the carbohydrate by reductive amination. The primary amine of a fluorescent tag and the C-1 aldehyde of the reducing sugar react to form a Schiff's base, which is reduced to the mixed aryl/aliphatic secondary amine by sodium cyanoborohydride, as shown in Fig. 1(a).

An important consideration about any labeling procedure is the efficiency and specificity of the derivatization step. To address this concern, experiments were performed to determine the labeling kinetics of maltotetraose. Experiments were performed using increasing labeling times

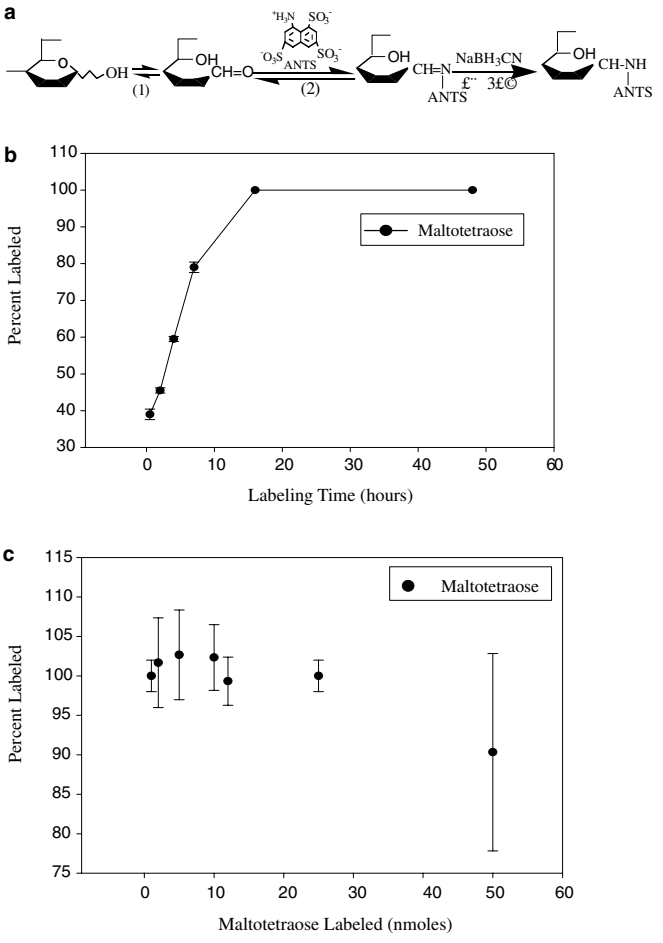
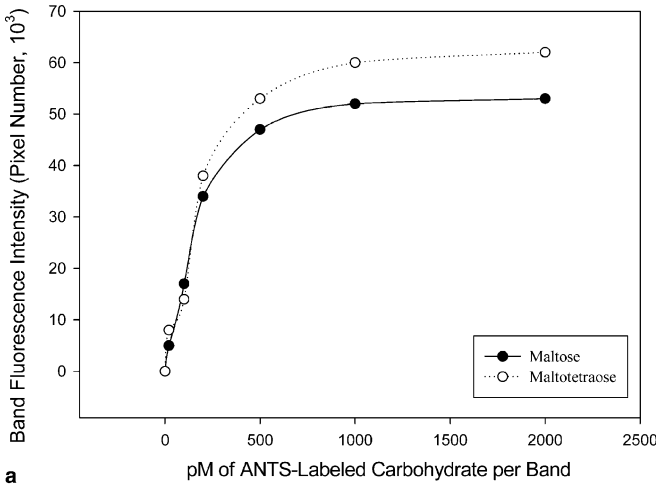
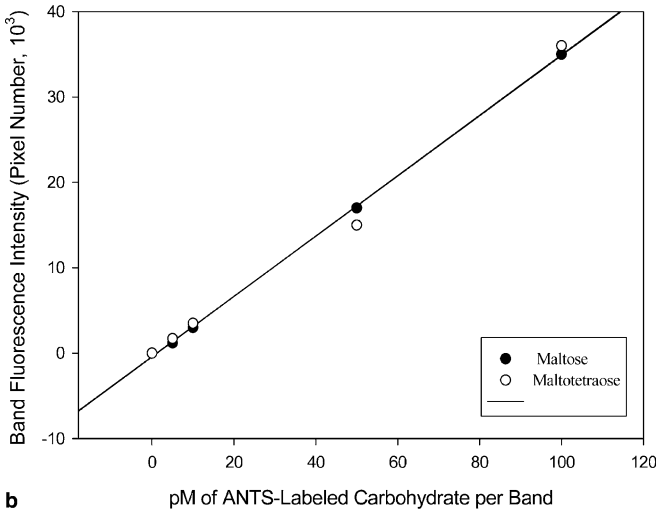


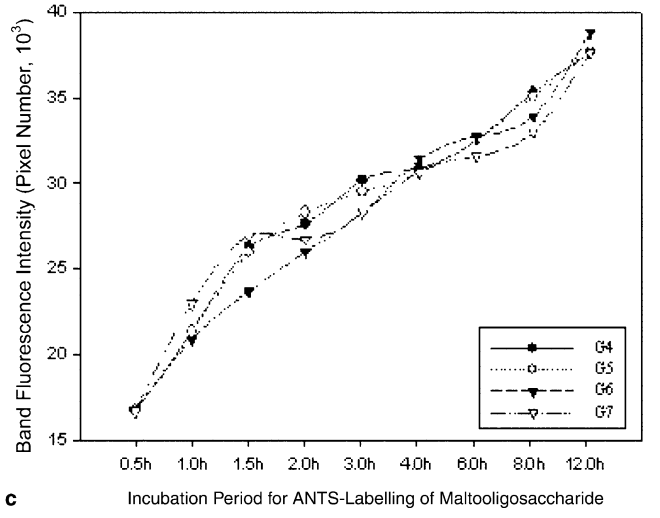
Fig. 1. FACE labeling chemistry. (a) The fluorophore labeling reaction with ANTS forming the Schiff's base and reduction by $NaBH_3CN$. (b) The labeling kinetics of 2 nmol of maltotetraose using 5 μL of 0.15 mol/L ANTS and 5 μL of 1.0 mol/L $NaBH_3CN$ at 37 °C. (c) The percent labeling efficiency of increasing amounts of maltotetraose with the same concentrations of ANTS and $NaBH_3CN$ for 16 h.



a



b



c

Fig. 2. Band fluorescence intensity as a function of carbohydrate concentration and malto-oligosaccharide length. ((a) and (b)) The relationship between band intensity and carbohydrate concentration was determined. Band fluorescence intensities of serial dilutions of maltose and maltotetraose were calculated and related to carbohydrate concentration for triplicate samples ($r^2 = 0.96140$). (c) Nonpreferential ANTS labeling of malto-oligosaccharides of various lengths was demonstrated. Aliquots of the malto-oligosaccharide were derivatized under identical conditions for 0.5–12 h. The oligosaccharide concentration of the maltotetraose (G4) through maltoheptaose (G7) demonstrated an ANTS-labeling rate independent of oligomer length.

(Fig. 1(b)) and increasing amounts of maltotetraose (Fig. 1(c)). When labeling relatively small amounts of maltotetraose, fluorophore labeling by reductive amination is very efficient, with >80% of the maltotetraose labeled in 6 h, and >95% labeled after 16 h (Fig. 1(b)). However, if the total amount of maltotetraose in the labeling reaction

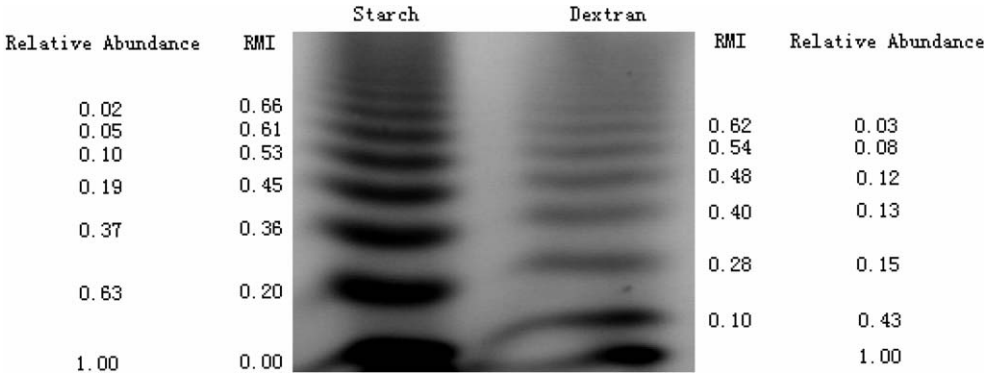


Fig. 3. FACE results for the ANTS-labeled oligosaccharides. The relative abundances of the individual oligoglucosides are indicated. Relative abundance was calculated using the linear regression ($r^2 = 0.96140$) derived from known concentrations of maltopentaose (Fig. 2(c)). The RMI of oligosaccharides derived from wheat starch and dextran were assigned unit values. The maltose front, RMI is 0.00.

is >25 nmol, labeling efficiency may decline (Fig. 1(c)). These results may help explain the reports of low labeling efficiency using reductive amination by alternative carbohydrate analytical techniques that require the labeling of nanomole or even micromole amounts of carbohydrate for detection.

3.2. Electrophoretic band intensity correlates with carbohydrate concentration

The sensitivity and quantitative limits of the methodology were determined by electrophoretic analysis of serial dilutions of the maltose and maltopentaose standards. At replicate concentrations less than 5 pmol/L, considerable variation in fluorescence intensities was recorded, although as little as 2 pmol/L/band could be seen visually. It shown that the relationship between fluorescence band intensity and carbohydrate concentration remained constant. That is, the relationship remained linear in the range of 5–100 pmol/L, with a decrease in sensitivity at higher carbohydrate concentrations (Fig. 2(a) and (b)).

A time course derivatization of the malto-oligosaccharide standard for 0.5–12 h indicated that ANTS labeling of the single terminal aldehyde per malto-oligosaccharide chain occurred without bias to malto-oligosaccharide length (Fig. 2(c)). That is, no one chain length was derivatized more readily than any other chain length. The relative abundance of all ANTS-labeled malto-oligosaccharides, as indicated by band fluorescence intensity, remained constant at all time points tested throughout the incubation period. Therefore, fluorescence band intensity is a direct measure of the relative abundance of individual oligosaccharide moieties in a heterogeneous sample.

3.3. Electrophoretic resolution of ANTS-labeled oligosaccharides

The graded diminished intensity of wheat starch and dextran bands with an increase in oligomer length is indicative of true random hydrolysis of this homogeneous substrate. The electrophoretic resolution of the ANTS-labeled oligosaccharides derived from the acid-hydrolyzed starch was sufficient to easily distinguish monomeric maltose through a 8-unit oligomer of glucose (Fig. 3).

The distances migrated by oligosaccharides of equal length derived from the hydrolysis of wheat starch and dextran illustrate the subtle effect of glycosidic linkage type on electrophoretic movement through the gel. The α-1,6-oligoglucosides of dextran, described as flexible coils, migrated at a slightly accelerated rate compared to the α-1,4-oligoglucosides of equal length derived from wheat starch, which are described as flexible helices (Kennedy & White, 1983). These RMI differences show the influence of hydroxyl positions on migration rate.

4. Conclusions

In a relatively short time, the FACE method has shown great potential as a flexible and reliable tool for performing routine, as well as, specialized carbohydrate analysis. As we have shown, the main advantages of this method are the sensitivity, the high resolving power of the separation, the ability to use essentially the same protocol for a variety of different applications, and the overall ease of using a familiar medium: gel electrophoresis. The ability of the gel system to analyze oligosaccharides without the need for separation of charged and uncharged oligosaccharides simplifies both profiling and sequencing. The gel format makes it very easy to compare the positions of oligosaccharides in different samples, as fully exploited in the FACE sequencing strategy. The ability to analyze multiple samples on the same gel makes this an ideal system for performing routine carbohydrate analysis such as often required in process development and quality control laboratories.

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