Optimization of Fluorophore-Assisted Carbohydrate Electrophoresis

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Fluorophore-assisted carbohydrate electrophoresis (FACE) is a simple and inexpensive method for separating saccharides. Oligosaccharides were tagged with the charged fluorophore 8-aminonaphthalene-1,3,6-trisulfonate (ANTS), and the reductive amination reactions were essentially complete after approximately 16 h under the given experimental conditions. Saccharide-ANTS adducts were then separated by electrophoresis on 32% C_{ACR}/2.4% C_{BIS} polyacrylamide gel at alkaline pH. This technique doesn't require sophisticated instrumentation and highly trained personnel.

Key words: Fluorophore-Assisted Carbohydrate Electrophoresis, Optimization, Oligosaccharide Derivatives

Introduction

The structural specifics and subtleties of (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 the 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 on the basis of size (Huang *et al.*, 2005, 2007; Huang and Zhang, 2007). 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 the polyacrylamide gel electrophoretic size 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 (Huang and Mei, 2006; Huang and Zhang, 2005, 2007).

However, the previous methods do not describe the determination limits of FACE. In this report, we discuss the determination limits of FACE in detail.

Results and Discussion

Electrophoretic band intensity correlates with carbohydrate concentration

The sensitivity and quantitative limits of the methodology are determined by electrophoretic analysis of serial dilutions of the glucose and laminaripentaose standards. At replicate concentrations of less than 5 pmol/L, considerable variation in fluorescence intensities was recorded, although as little as 2 pmol/L could be seen. This showed that the relationship between fluorescence band intensity and carbohydrate concentration remained constant. That is, the relationship remained linear in the range of 5 to 100 pmol/L, with a decrease in sensitivity at higher carbohydrate concentrations (Figs. 1A, B). Moreover, no chain length was derivatized more readily than any other chain length. The relative abundance of all ANTS-labeled glucooligosaccharides, 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 heterogenous sample.

Determination limits of FACE

Polyacrylamide gel electrophoresis was employed for the analytical separation of ANTS-derivatized β-1,3-oligoglucosides. Compared with the molecular masses of proteins and DNA, oligosaccharides are generally in the range of a few 1000

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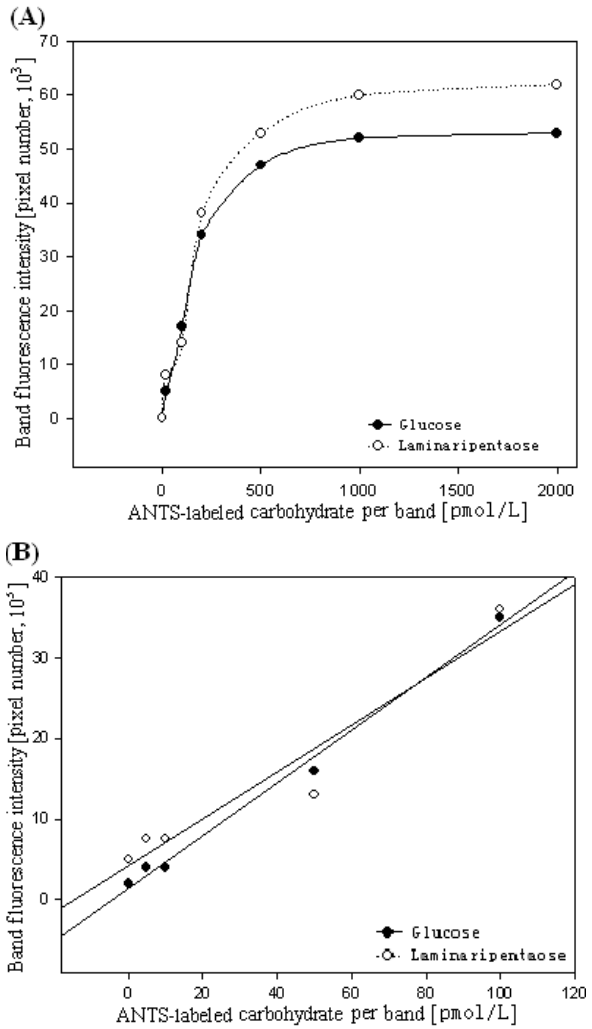

Fig. 1. Relationship between band intensity and carbohydrate concentration. Band fluorescence intensities of serial dilutions of glucose and laminaripentaose were calculated and related to carbohydrate concentration for triplicate samples ($r^2 = 0.96140$). (A) When the carbohydrate concentration was more than 100 pmol/L, the relationship between fluorescence band intensity and concentration was not linear, with a decrease in sensitivity at higher carbohydrate concentration. (B) The relationship between fluorescence band intensity and carbohydrate concentration remained linear in the range of 5 to 100 pmol/L.

and monosaccharides are in the range of 180–300. Therefore, in order to separate oligosaccharides, very high percentage polyacrylamide gels must be employed. In our experiments, saccharides of various lengths were derivatized and the saccharide-ANTS adducts were separated on 18.6% C_{ACR}/1.4% C_{BIS} (Fig. 2A); 32% C_{ACR}/2.4% C_{BIS} (Fig. 2B); and 37.2% C_{ACR}/2.8% C_{BIS} (Fig. 2D) polyacrylamide gels at alkaline pH value, respectively. It was indicated that the separation effects were different, as shown in Fig. 2. The separation effect was the best when the saccharide-ANTS adducts were separated on 32% C_{ACR}/2.4% C_{BIS} polyacrylamide gel (Fig. 2B). Therefore, the optimal gel content for FACE is about 30% total acrylamide. Saccharides of various lengths can easily be distinguished from one another because the ANTS adducts of the smaller saccharides have greater electrophoretic mobilities than those of the larger saccharides.

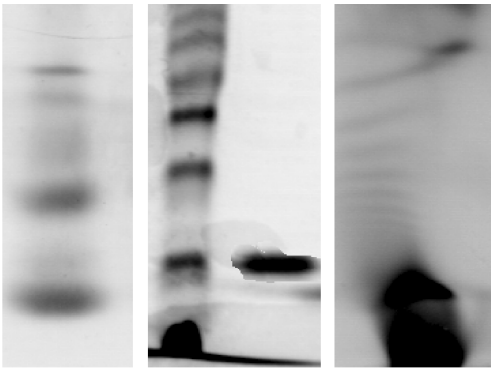

Fig. 2. Separation of oligosaccharide-ANTS adducts (1 μmol) of various lengths on polyacrylamide gel at alkaline pH. (A) 18.6% C_{ACR}/1.4% C_{BIS} polyacrylamide gel; (B) 32% C_{ACR}/2.4% C_{BIS} polyacrylamide gel; (C) ANTS-labeled glucose as marker; (D) 37.2% C_{ACR}/2.8% C_{BIS} polyacrylamide gel.

Materials and Methods

Chemicals

Glucose, laminaripentaose, *N,N′*-methylenebisacrylamide, β-1,3-glucan, *N,N,N′,N′*-tetramethylethylenediamine (TEMED), ammonium persulfate, 8-aminonaphthalene-1,3,6-trisulfonate (ANTS) and sodium cyanoborohydride were purchased from Sigma-Aldrich Chemical Company.

Partial acid hydrolysis of glucan particles

To prepare hydrolysates, 50–100 mg of glucan particles were suspended at a concentration of 5 mg/mL in trifluoroacetic acid, heated for a period of time in 25-mL reactiflasks, cooled, and centrifuged at 160 × *g* for 10 min at 25 °C. Superna-

tant fractions from 10 reactiflasks were pooled and the residual trifluoroacetic acid was removed by rotoevaporation at 35–40 °C in a silanized round-bottom flask. The sample was lyophilized.

ANTS labeling of oligosaccharides

The dried oligosaccharides sample was suspended in 5.0 mL 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(hydroxymethyl)aminomethane (Tris)-HCl, pH 6.8, containing 20% glycerol] and stored at −70 °C.

Electrophoresis of ANTS-labeled oligosaccharides

The resolving gel was 32% acrylamide/2.4% bisacrylamide in a $140 \times 160 \times 0.75$ 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 and 5 μL of ammonium persulfate and TEMED, respectively, for every 6 mL of stacking gel. The running buffer and the gel buffer were 0.025 mol/L Tris/0.192 mol/L glycine (pH 8.4) and 0.42 mol/L Tris (pH 8.5), respectively. Electrophoresis was run at a constant current of 15 mA for 6 h in a cooled buffer system.

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 UV illumination (365 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 apparatus at a resolution of 300 dpi; the images were inverted (inverse pixels) using Adobe Photoshop 4.0. 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).

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