

Interactions of carbohydrates and proteins by fluorophore-assisted carbohydrate electrophoresis

GANG-LIANG HUANG*, XIN-YA MEI and PENG-GEORGE WANG

State Key Laboratory of Microbial Technology, Shandong University, Jinan, Shandong 250100,
People's Republic of China

*Corresponding author (Fax, 86-531-88366078; Email, hgl226@126.com)

A sensitive, specific, and rapid method for the detection of carbohydrate-protein interactions is demonstrated by fluorophore-assisted carbohydrate electrophoresis (FACE). The procedure is simple and the cost is low. The advantage of this method is that carbohydrate-protein interactions can be easily displayed by FACE, and the carbohydrates do not need to be purified.

[Huang G-L, Mei X-Y and Wang P-G 2006 Interactions of carbohydrates and proteins by fluorophore-assisted carbohydrate electrophoresis; *J. Biosci.* **31** 219–222]

1. Introduction

Molecular recognition by specific targets is at the heart of the life processes. In recent years, it has been shown that the interactions between carbohydrates and proteins mediate a broad range of biological activities, starting from fertilization, embryogenesis, and tissue maturation, and extending to such pathological processes as tumour metastasis.

Fluorophore-assisted carbohydrate electrophoresis (FACE) is a high-resolution polyacrylamide gel electrophoretic procedure that separates oligosaccharides on the basis of size (Jackson 1990, 1994). Individual carbohydrate moieties are tagged at the terminal aldehyde with the highly charged fluorophore 8-aminonaphthalene-1,3,6-trisulphonate (ANTS), which imparts a uniformly strong negative charge to each oligosaccharide or monomeric reducing sugar and enables the use of 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 (Jackson 1990; Stack *et al* 1992).

In this paper, we describe the application of FACE for determining the relative abundance of oligomannosides released by acid hydrolysis of mannan from *Saccharomyces cerevisiae*. It also demonstrates that the interactions of carbohydrates and proteins are amenable to FACE analysis.

2. Materials and methods

2.1 Materials

Mannose, mannotetraose, mannopentaose, mannohexaose, mannoheptaose, *Canavalia ensiformis* Con A, mannan from *Saccharomyces cerevisiae*, *N,N'*-methylenebisacrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persulphate, ANTS and sodium cyanoborohydride were purchased from Sigma-Aldrich Chemical Company (USA). SigmaGel gel analysis software was purchased from Statistical Package for the Sciences (SPSS) Inc., Chicago, Ill, USA.

2.2 Partial acid hydrolysis of mannan

To prepare hydrolysates, 50 to 100 mg of mannan were suspended at a concentration of 1% hydrochloric acid, heated at 60°C for 110 min in 25 ml rectiflasks cooled, and centrifuged at 160 g for 10 min at 25°C. Supernatant fractions from 10 rectiflasks were pooled and the residual hydro-chloric acid was removed by rotoevaporation at 35°C to 40°C in a silanized round bottom flask. The sample was lyophilized.

Keywords. Abundance; 8-aminonaphthalene-1,3,6-trisulphonate; fluorophore-assisted carbohydrate electrophoresis; interaction of carbohydrates and proteins

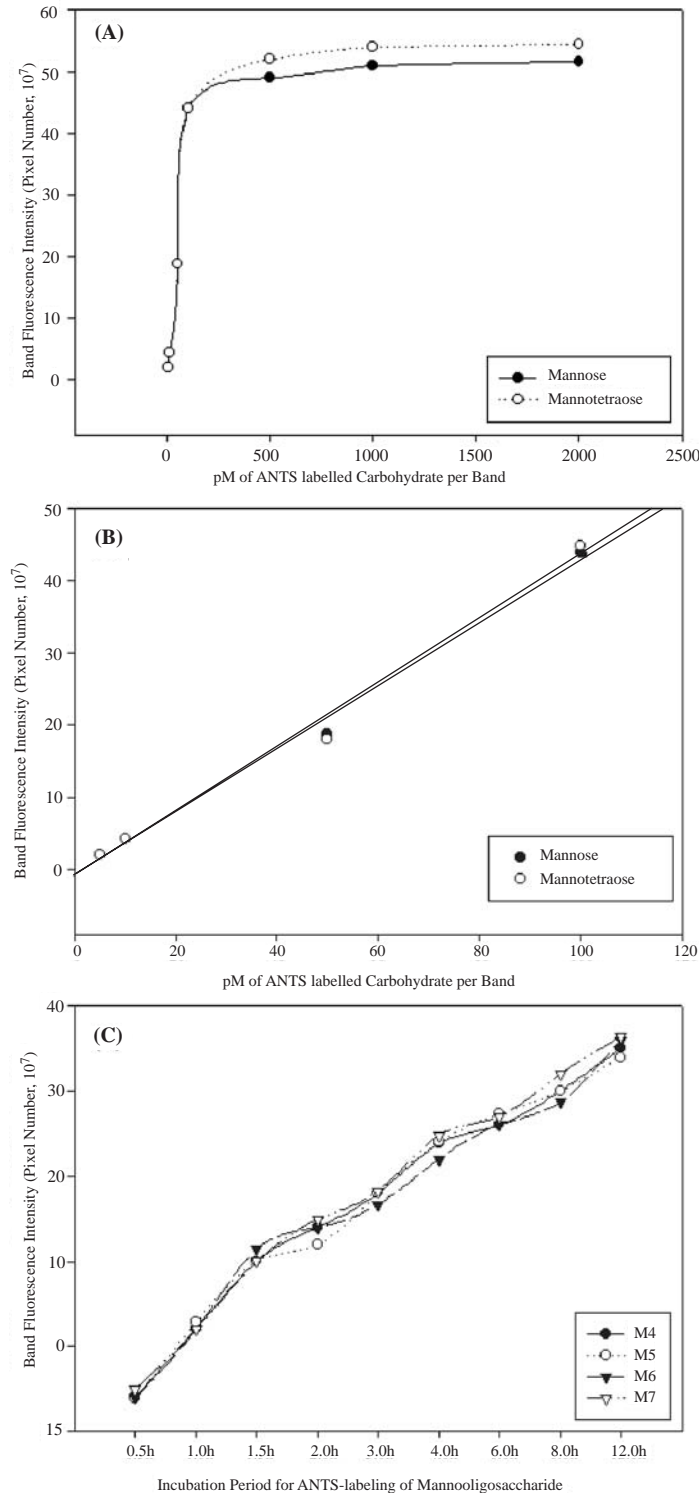


Figure 1. Band fluorescence intensity as a function of carbohydrate concentration and manno oligosaccharide length. (A, B) The relationship between band intensity and carbohydrate concentration was determined. Band fluorescence intensities of serial dilutions of mannose and mannotetraose were calculated and related to carbohydrate concentration for triplicate samples ($r^2= 0.96140$). (C) Nonpreferential ANTS labelling of manno oligosaccharides of various lengths was demonstrated. Aliquots of the manno oligosaccharide were derivatized under identical conditions for 0.5 to 12 h. The oligosaccharide concentration of the maltotetraose (M4) through mannoheptaose (M7) demonstrated an ANTS-labelling rate independent of oligomer length.

2.3 Binding of oligomannosides to Con A

Oligomannoside-protein binding studies were carried out by incubating the two components in 50 mM Tris-HCl, pH 7.5, 0.15 mM NaCl, 1 mM MnCl₂, 1 mM MgCl₂, and 1 mM CaCl₂ at room temperature for 30 min.

2.4 ANTS labelling of oligomannoside-Con A complex

The 100 nmol oligomannoside-Con A complex was tagged with ANTS as described elsewhere (Jackson 1990). The dried oligomannoside-Con A complex 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 M sodium cyanoborohydride in dimethyl sulphoxide 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 mM Tris-HCl, pH 6.8, containing 20% glycerol), and stored at -70°C.

2.5 Electrophoresis of ANTS-labelled oligomannosides and oligomannoside-Con A complex

The electrophoretic method used was an adaptation of that previously reported (Jackson 1990). The resolving gel was 32% acrylamide-2.4% bisacrylamide in a 140 × 160 × 0.75 mm glass cassette. For every 35 ml of resolving gel, 150 µl of 10% ammonium persulphate (APS) and 15 µl of TEMED were added. The stacking gel was made of 8% acrylamide -0.6% bisacrylamide containing 50 µl of 10% APS and 5 µl of TEMED, respectively, for every 6 ml of stacking gel. The running and the gel buffers were composed of 0.025 M Tris base -0.192 M glycine (pH 8.4) and 0.42 M Tris base (pH 8.5), respectively. Electrophoresis was run at a constant current of 15 mA for 6 h in a cooled buffer system.

2.6 Visualization, photography, and image analysis

For visualization of the ANTS-labelled oligosaccharides, the gel was removed from the glass cassette and placed onto the surface of a light box with ultraviolet (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 with an exposure time of 3 to 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 analysed 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).

3. Results and discussion

3.1 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 mannose and mannotetraose standards. At replicate concentrations less than 5 pM, considerable variation in fluorescence intensities was recorded, although as little as 2 pM/band could be seen visually. It shows 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 pM, with a decrease in sensitivity at higher carbohydrate concentrations (figure 1A, B).

A time course derivatization of the manno oligosaccharide standard for 0.5 to 12 h indicated that ANTS labelling of the single terminal aldehyde per manno oligosaccharide chain occurred without bias to manno oligosaccharide length (figure 1C). That is, no one chain length was derivatized more readily than any other chain length. The relative abundance of all ANTS-labelled manno 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.

3.2 The interaction of oligomannosides and Con A by FACE

Because of the very high percentage of acrylamide used in the FACE system, only carbohydrates are able to move into the gel, whereas high molecular weight molecules such as proteins are not. Consequently, if a carbohydrate binds to a protein, its electrophoretic mobility should be retarded. To determine whether this assumption could be exploited for quantitative analysis of carbohydrate-protein interactions, we examined a lectin-carbohydrate model. The migration of saccharide-ANTS adducts in a polyacrylamide gel is shown in figure 2. Left lane is oligomannosides control incubated without any Con A. The four bands in left lane are trisaccharide, disaccharide, monosaccharide, and ANTS respectively, from top to bottom. Right lane is oligomannosides incubated with Con A. The four bands in right lane are trisaccharide, disaccharide, monosaccharide, and ANTS respectively, from top to bottom. This indicates that the retardation was observed when the mixture of oligomannosides was incubated with less or more Con A. These results

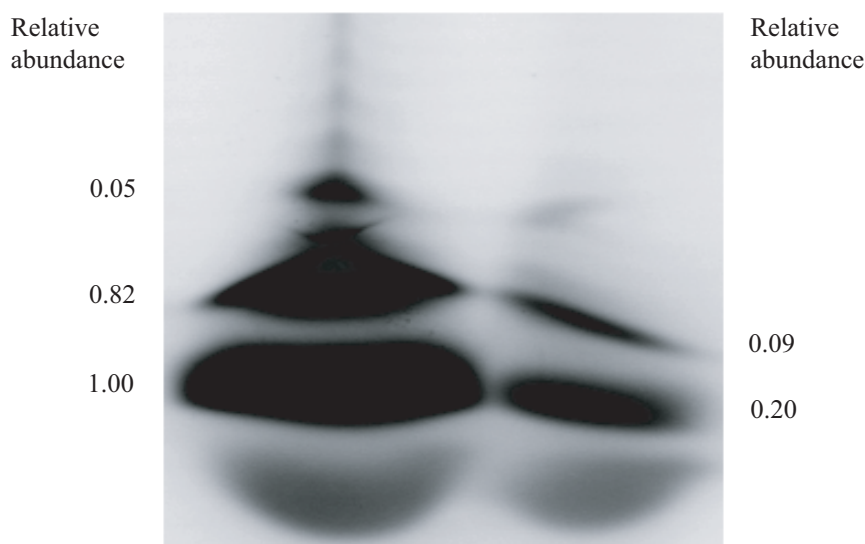


Figure 2. FACE assay the interaction of oligomannosides and Con A. The relative abundances of the individual oligomannosides are indicated. Relative abundance was calculated using the linear regression ($r^2=0.96140$) derived from known concentrations of mannose-6-phosphate (refer to figure 1C).

demonstrate the feasibility of this electrophoretic approach for study of carbohydrate-protein interactions.

References

Jackson P 1994 High-resolution polyacrylamide gel electrophoresis of fluorophore-labeled reducing saccharides; *Methods Enzymol.* **230** 250–265

Jackson P 1990 The use of polyacrylamide-gel electrophoresis for the high-resolution separation of reducing saccharides labelled with the fluorophore 8-aminonaphthalene-1,3,6-trisulphonic acid; *Biochem. J.* **270** 705–713

Stack R J and Sullivan M T 1992 Electrophoretic resolution and fluorescence detection of N-linked glycoprotein oligosaccharides after reductive amination with 8-aminonaphthalene-1,3,6-trisulphonic acid; *Glycobiology* **2** 85–92

MS received 21 December 2005; accepted 13 April 2006

ePublication: 26 April 2006

Corresponding editor: DIPANKAR CHATTERJI