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# Fluorophore-Assisted Carbohydrate Electrophoresis as Detection Method for Carbohydrate–Protein Interactions

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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 (oligo)saccharides 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 is not biased by (oligo)saccharide length. Therefore, band fluorescence intensity is directly related to the relative abundance of individual (oligo)saccharide moieties in heterogeneous sample. In the same time, it also indicates that FACE can be used to investigate the interactions of carbohydrates and proteins.

**Index Entries:** Fluorophore-assisted carbohydrate electrophoresis; mannan; 8-aminonaphthalene-1,3,6-trisulfonate; concanavalin A; carbohydrate–protein interactions.

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## Introduction

With the rapid advancement of glycobiology, more and more important biologic processes have been proved to depend on carbohydrate-protein interactions. The carbohydrates on glycoproteins have been shown to be important in the regulations of bioactivity *(1)*, pharmacokinetics *(2)*, immunogenicity *(3)*, stability *(4)*, and efficacy *(5)*.

Electrophoresis is one of the most common and powerful technologies for the separation and identification of mixtures of proteins and nucleic acids. By contrast, because many carbohydrates are not charged, the development of carbohydrate electrophoresis has lagged behind. To overcome this problem, fluorophore-assisted carbohydrate electrophoresis (FACE) *(6)* was developed. FACE is based on the attachment of fluorescent dyes to the reducing end of carbohydrate followed by high-resolution separation on polyacrylamide slab gels. FACE can be used to perform a wide variety of analyses for carbohydrates from multiple sources including glycoproteins, glycolipids, plant and bacterial polysaccharides, as well as glycosaminoglycans.

In the present study, we demonstrated that FACE could be used for sensitive detection of concanavalin A (Con A)–oligomannoside interaction.

## Materials and Methods

### Chemicals

8-Aminonaphthalene-1,3,6-trisulfonate (ANTS) was purchased from Eugene. Fluorescein isothiocyanate (FITC)-labeled and unlabeled *Canavalia ensiformis* (Con A) were purchased from Vector. All other materials were purchased from Sigma or Aldrich.

### Nonspecific Acidic Hydrolysates of Mannan

Mannan (10 μg) was added to $ddH_2O$ (1 μL). After 5 min, mannan dissolved in $ddH_2O$ with the help of ultrasonic. Then, HCl (5 μL, 10 mol/L) was added to the solution, and the resulting mixture was stirred to form the acid solution of mannan (0.05 mol/L of HCl, 1% mannan). This solution was referred to as M1. The acid solutions of mannan and amylum (0.1 mol/L of HCl, 1% polysaccharide) were prepared in a similar manner. These two kinds of solution were named M2 and Amy, respectively. Three 1-μL EP cuvets were kept in the water for 2 h at 65°C. Finally, these three kinds of solution were frozen to dry under subatmospheric pressure and were kept at 4°C.

### Detection of Hydrolyzed Mannan Polymorphisms by FACE

The reducing ends of the hydrolyzed mannan (M1, M2, and Amy) were attached to ANTS, which conferred negative charge on the polysaccharide *(7)*. With the proper condition of electrophoresis, the hydrolyzed

polysaccharide polymorphisms were displayed on the gel, and the ANTS-glucose and ANTS-maltose were used as control.

### *Detection of Hydrolyzed Polysaccharide–Con A Interactions by FACE*

Con A (1 μg) was added to and dissolved in 50 μL of buffer solution of oligosaccharide–Con A interaction (50 μmol/L of Tris-HCl, pH 7.5; 0.15 μmol/L of NaCl; 1 μmol/L of $MnCl_2$; 1 μmol/L of $MgCl_2$; 1 μmol/L of $CaCl_2$) in a 0.1-μL EP cuvet to give the Con A solution (20 μg/mL).

#### Experiment 1

The hydrolyzed mannan (5 μL) whose reducing end was attached to ANTS was added to the Con A solution (15 μL). The mixed solution, named M-C1, was kept at room temperature for 2 h to allow the hydrolyzed mannan and Con A to interact, and then it was stored at 4°C.

Next, the hydrolyzed mannan (5 μL) whose reducing end was attached to ANTS was added to the buffer solution (15 μL) in a 0.1-mL EP cuvet. The mixed solution was stored at 4°C. It was used as the control for experiment 1 and was called control 1.

#### Experiment 2

The hydrolyzed mannan (1 mg) was added to the Con A solution (15 μL). The mixed solution was kept at room temperature for 2 h to allow the hydrolyzed mannan and Con A to interact. ANTS was added to the mixed solution to react with the reducing end of the oligomannosides. This mixed solution was called M-C2.

Next, the hydrolyzed mannan (1 mg) was added to the butter solution (15 μL). ANTS was added to the mixed solution to react with the reducing end of the oligomannosides. This mixed solution was used as the control for experiment 2 and was called control 2.

## Results and Discussion

### *Detection of Hydrolyzed Polysaccharide Polymorphism by FACE*

We first investigated the hydrolysis of polysaccharide using FACE to identify an optimal condition for polysaccharide hydrolysis. Monosaccharide residues of mannan and amylose were mainly linked by an α-glucosidic bond, which is prone to breakage by the acid hydrolysis. As a result, gentle hydrolytic condition was necessary. To determine the number of mannose units of the hydrolyzed mannan, amylum was hydrolyzed to form an oligosaccharide marker, which contained 2–10 glucose units. At the same time, glucose was used as a monosaccharide marker.

Figures 1 and 2 show that 0.05 or 0.1 mol/L of HCl could hydrolyze mannan, although more monosaccharide was produced at these conditions. Because of the random hydrolysis, mannan was prone to produce a ladder, which contained 2–10 or more units of mannose. The bands of the

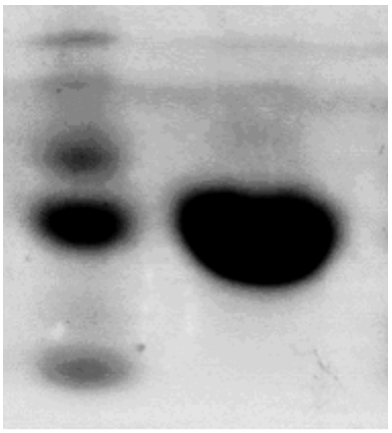


Fig. 1. Electrophoresis pattern of mannooligosaccharides and glucose. Lane 1, mannooligosaccharides; lane 2, glucose.

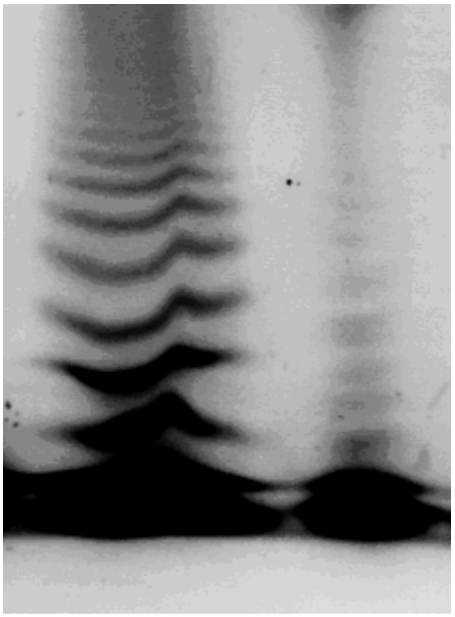


Fig. 2. Electrophoresis pattern of amylum and mannooligosaccharides. Lane 1, amylum; lane 2, mannooligosaccharides.

saccharides in Fig. 2 were more abundant than those in Fig. 1. Therefore, we used 0.05 mol/L of HCl and 1% polysaccharide for 2 h at 65°C as the hydrolytic condition of mannan. Figure 2 shows that the hydrolysis of amylum was more random and easier than that of mannan. In FACE of amylum hydrolysate, the bands that corresponded to oligosaccharides of 2–10 glu-

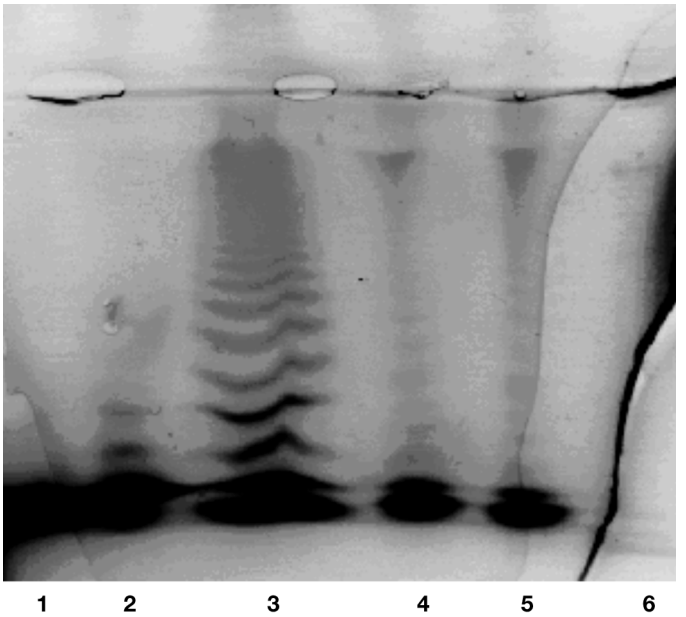


Fig. 3. FACE of ANTS–mannooligosaccharide–Con A interactions. Lane 1, glucose; lane 2, maltose; lane 3, amylum hydrolysate; lane 4, M-C1; lane 5, control 1; lane 6, FITC–Con A.

cose units clearly formed a ladder. The relative amounts of disaccharide, trisaccharide, tetrasaccharide, and other oligosaccharides in amylum hydrolysate were more than the amount in mannan hydrolysate. This result suggests that amylum hydrolysate can be used as a molecular mass marker in oligosaccharide analysis.

### *Possible Influence of ANTS on Carbohydrate-Protein Interactions*

Figure 3 shows that FACE of M-C1 and Control 1 were the same; the ANTS-oligosaccharides did not interact with Con A. The unreacted Con A was kept in the gel because of its large size. The result that ANTS-mannooligosaccharides did not bind with Con A indicated that ANTS could affect the structure of Con A because of its negative charge. The change in the Con A structure will directly affect the biologic function of Con A. Thus, ANTS influenced the carbohydrate-protein interactions. This suggests that avoiding the influence from ANTS is very important for the detection of carbohydrate-protein interactions.

### *Detection of Hydrolyzed Polysaccharide–Con A Interactions by FACE*

To avoid the influence from ANTS, we incubated the hydrolyzed mannan with Con A first, followed by attachment to ANTS. Using this method, we obtained the data presented in Fig. 4, which shows a differential analy-

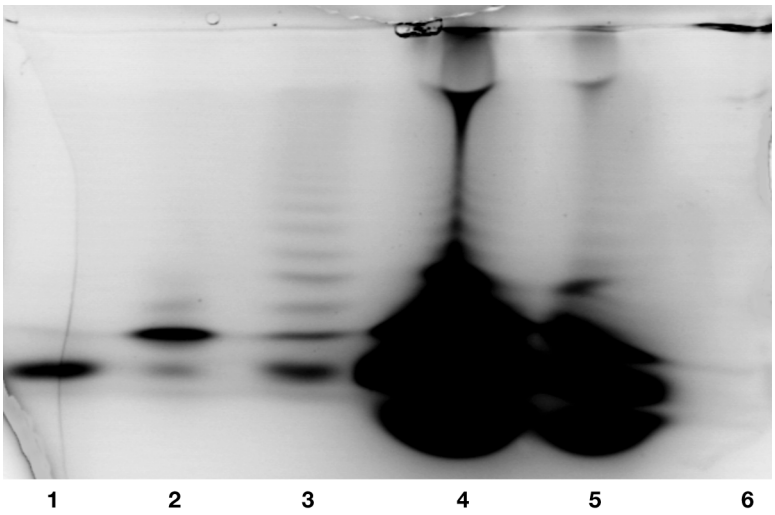


Fig. 4. FACE of mannooligosaccharide–Con A interactions. The mannooligosaccharides were first incubated with Con A and then attached to ANTS. Lane 1, glucose; lane 2, maltose; lane 3, amylum; lane 4, control 2; lane 5, M-C2; lane 6, FITC–Con A.

sis of oligosaccharide-protein interactions. According to Fig. 3, lanes 4 and 5 in Fig. 4 represent FACE of the mannooligosaccharide mixture before and after incubation with Con A, respectively. The fact that all oligosaccharides decreased obviously after incubation with Con A indicated that the interactions between mannooligosaccharides and Con A occur in many sites and are not specific. This result is consistent with the carbohydrate-binding character of Con A and also with the view that the antigenic determinant of mannan is at its nonreducing end.

## Conclusion

The data taken together indicate the feasibility of FACE for studying carbohydrate-protein interactions; and the procedure is rapid, convenient, and the cost is low.

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