# Analysis of Interactions between Carbohydrates and Proteins Using Fluorescent Labeling and SDS-PAGE

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A rapid method for the analysis of carbohydrate-protein interactions by using fluorescent labeling and SDS-PAGE was developed. The *N*-acetyl-$\beta$-D-glucosamine-WGA complex and $\alpha$-D-mannose-Con A complex were labeled with 8-aminonaphthalene-1,3,6-trisulfonate (ANTS). The protein band displaying fluorescence with ultraviolet illumination was seen after SDS-PAGE.

*Key words:* Carbohydrate-Protein Interactions, Fluorescent Labeling, SDS-PAGE

## Introduction

Carbohydrate-binding proteins and their glycoconjugate ligands play significant roles in a number of critical biological processes. Immune response, viral membrane fusion, glycoprotein homeostasis, and signaling involve carbohydrate-binding protein mediation at key steps (Lee *et al.*, 2002). A more detailed understanding of the exact nature of carbohydrate-protein interactions is expected to render them attractive therapeutic targets. To facilitate these biochemical investigations, glycobiologists require tools that will enable the simultaneous identification of carbohydrate-binding proteins and oligosaccharide structures to which they bind. Details of carbohydrate-protein interactions have been investigated mainly by biophysical and biochemical approaches, including X-ray crystallography, NMR spectroscopic studies of carbohydrate-protein complexes, glycoarray (Huang *et al.*, 2005; Huang and Zhang, 2006), and fluorophore-assisted carbohydrate electrophoresis (FACE) (Huang and Mei, 2006; Huang *et al.*, 2007). FACE is a high-resolution polyacrylamide gel electrophoretic procedure that separates oligosaccharides on the basis of size. Individual carbohydrate moieties are labeled at the terminal aldehyde group 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.

## Results

We studied the interactions of carbohydrates and proteins by using fluorescent labeling and SDS-PAGE. It was emphasized that the present method differs from FACE. That is to say, after we used a fluorophore to label carbohydrate-protein complexes, the fluorescent labeled carbohydrate-protein complexes were analyzed by SDS-PAGE.

We incubated wheat germ agglutinin (WGA) with *N*-acetyl-$\beta$-D-glucosamine and concanavalin A (Con A) with $\alpha$-D-mannose, respectively, followed by ANTS labeling, conventional SDS-PAGE, and detection of fluorescent protein bands (see Figs. 1 and 2). The ANTS-labeled WGA (or Con A), and WGA (or Con A to exclude autofluorescence) were used as controls. We found that the fluorescence intensity of the ANTS-labeled *N*-acetyl-$\beta$-D-glucosamine-WGA complex (or ANTS-labeled $\alpha$-D-mannose-Con A complex) was higher than the one of ANTS-labeled WGA (or ANTS-labeled Con A). So, it was confirmed that WGA (or Con A) interacts with *N*-acetyl-$\beta$-D-glucosamine (or $\alpha$-D-mannose). WGA is not blood group-specific but has an affinity for *N*-acetyl-$\beta$-D-glucosaminyl residues and *N*-acetyl-$\beta$-D-glucosamine oligomers. Con A is not blood group-spe-

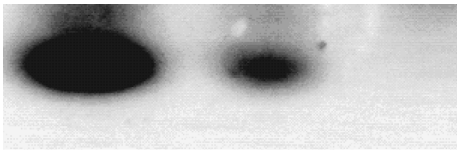


Fig. 1. SDS-PAGE-UV profile of the ANTS-labeled *N*-acetyl-$\beta$-D-glucosamine-WGA complex. Lane 1, ANTS-labeled *N*-acetyl-$\beta$-D-glucosamine-WGA complex; lane 2, ANTS-labeled WGA; lane 3, WGA.


Fig. 2. SDS-PAGE-UV profile of the ANTS-labeled $\alpha$-D-mannose-Con A complex. Lane 1, Con A; lane 2, ANTS-labeled $\alpha$-D-mannose-Con A complex; lane 3, ANTS-labeled Con A.

cific but has an affinity for terminal α-D-mannosyl and α-D-glucosyl residues. They are both sugar-binding proteins. Therefore, our results are consistent with the known facts.

In conclusion, this work shows an efficient methodology for the analysis of carbohydrate-protein interactions by using fluorescent labeling and SDS-PAGE. We believe that this method, together with the efficient methods available for the synthesis of complex oligosaccharides, can become useful for the biological evaluation of carbohydrate-protein interactions.

## Materials and Methods

### *Materials*

*N,N′*-Methylenebisacrylamide, *N,N,N′,N′*-tetramethylethylenediamine (TEMED), ammonium persulfate, *N*-acetyl-β-D-glucosamine, α-D-mannose, 8-aminonaphthalene-1,3,6-trisulfonate (ANTS), sodium cyanoborohydride, concanavalin A (Con A) from *Canavalia ensiformis*, and wheat germ agglutinin (WGA) from *Triticum vulgaris* were purchased from Sigma-Aldrich Chemical Company (USA).

### *Binding of N-acetyl-β-D-glucosamine (or α-D-mannose) to WGA (or Con A)*

*N*-Acetyl-β-D-glucosamine-WGA (or α-D-mannose-Con A) binding studies were carried out by incubating the two components in 50 mmol/L tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.5, 0.15 mmol/L NaCl, 1 mmol/L $MnCl_2$, 1 mmol/L $MgCl_2$, and 1 mmol/L $CaCl_2$ at room temperature for 30 min.

### *ANTS labeling of N-acetyl-β-D-glucosamine-WGA (or α-D-mannose-Con A) complex*

The *N*-acetyl-β-D-glucosamine-WGA (or α-D-mannose-Con A) complex was labeled with ANTS as described elsewhere (Huang and Zhang, 2005, 2007). The dried *N*-acetyl-β-D-glucosamine-WGA (or α-D-mannose-Con A) complex 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-HCl, pH 6.8, containing 20% glycerol), and stored at −70 °C.

### *Visualization and photography*

For visualization of the ANTS-labeled *N*-acetyl-β-D-glucosamine-WGA (or α-D-mannose-Con A) complex, the gel was removed from the glass cassette and placed onto the surface of a light box with ultraviolet (UV) illumination (365 nm). The gel was 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 to 10 s. The photograph was 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.

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