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Structure–Function Relations of Carbohydrates by Neoglycolipid Arrays

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Abstract

The work presented herein is a new noncovalent glycoarray assembly method for microplates created by simply mixing together a carbohydrate and a tetradecylamine. α-D-Mannopyranoside, α-D-glucopyranoside, and α-D-galactopyranoside were utilized in model studies and product formations were detected by lectin binding. The method can be extended to study the steric hindrance effect of carbohydrate–protein interactions, namely the structure–function relations of carbohydrates.

Index Entries: Glycoarray; neoglycolipid; structure–function relations.

Introduction

Mammalian cell surfaces are coated with a layer of carbohydrate molecules attached to lipids and proteins, which is known as the glycocalyx. These often complex and heterogeneous glycans are involved in a diverse array of biological processes including inflammation, metastasis, cell–cell adhesion, and pathogen–cell interactions *(1,2)*. Identification of the specific carbohydrates involved in these processes is important to better understand cell–cell recognition at the molecular level and to aid the design of therapeutics and diagnostic tools.

Microarrays are becoming one of the most powerful technologies for the identification and biological evaluation of new drug candidates and targets. Few approaches have been developed thus far for the fabrication of glycoarrays. Wang et al. *(3)* found that nitrocellulose-coated glass slides

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can be used to immobilize microspots of carbohydrate polymers without covalent conjugation. Houseman and colleagues *(4–6)* exploited the Diels-Alder-mediated immobilization of carbohydrate–cyclopentadiene conjugates to a monolayer that presents benzoquinone groups displayed on a gold surface. Recently, Park and Shin *(7)* reported on the attachment of maleimide-linked carbohydrates to a glass slide coated with thiol groups. In addition, Fukui et al. *(8)* described microarrays of oligosaccharides displayed as neoglycolipids on nitrocellulose. Furthermore, Fazio et al. *(9)* first noncovalently immobilized the long-chain (14-carbon) aliphatic alkyne on the plastic surface and then carried out *in situ* conjugation of the azide forms of galactose and several neutral and sialic acids containing di- to tetrasaccharides.

In the present study, our goal was to develop a simple and efficient system to attach neoglycolipids to a microtiter plate to screen for their specific interactions with proteins.

Materials and Methods

Equipment and Chemicals

A Costar 96-well half-area high-binding polystyrene plate was purchased from Fisher. Fluorescein isothiocyanate (FITC)-labeled *Canavalia ensiformis* Con A was purchased from Vector. All other materials were purchased from Sigma or Aldrich.

Spectrophotometry

Emission spectra were used to corroborate and quantify epifluorescence images and to detect features outside the visible range. Spectra were collected with a Hitachi F-4500 fluorescence spectrophotometer illuminated by a 100-W Xe lamp. Spectra were typically taken at a scanning rate of 1200 nm/min with 2.5-nm excitation and emission slits and a 700-V photomultiplier tube voltage. Efforts were made to keep the samples dilute, so that their optical densities at 490 nm were less than or equal to 0.05. The fluorescence measurements were treated qualitatively. Absorbance spectra were recorded with a Hewlett-Packard 8453 UV-visible spectrophotometer.

Synthesis of Lipid-Linked Monosaccharides

α-D-Mannopyranoside (or α-D-glucopyranoside and α-D-galactopyranoside, 24 m*M*) was suspended in 5.0 mL of 20 m*M* tetradecylamine in acetic acid-water (10X) and freshly made 50 m*M* sodium cyanoborohydride in dimethyl sulfoxide (DMSO) and incubated at 37°C for 20 h. The sample was dried under nitrogen at 45°C and stored at –70°C.

Con A Assay

Wells containing lipid-linked monosaccharides were washed five times with dH_2O (100 mL). Each wash consisted of exchange between the

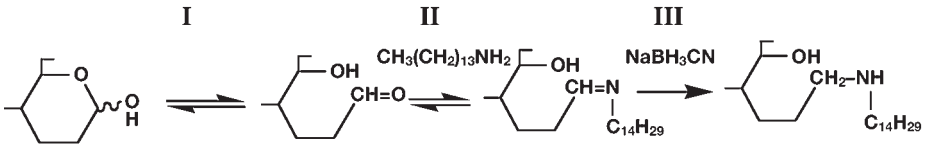

Fig. 1. Synthetic route of lipid-linked monosaccharides. Reagents and conditions: (I) CH_3COOH (10%); (II) $C_{14}H_{29}NH_2$; (III) $NaBH_3CN$, DMSO, 37°C, 20 h.

well and a multichannel pipetman five times to remove excess sodium cyanoborohydride and unreactive monosaccharides. All wells were then incubated with 10 m*M* HEPES buffer, pH 7.5/150 mmol/L of NaCl containing 1X bovine serum albumin (100 mL) (buffer A) for 1 h. Buffer A was then removed from the well and a solution of FITC–Con A in buffer A (10 mg/mL of buffer A; 100 mL) was incubated in the well for 1 h. Wells were then washed again with buffer A (100 mL) for 1 h. This was exchanged for fresh buffer A (100 mL) and the fluorescence was read at 535 nm.

Results and Discussion

Synthesis of Lipid-Linked Monosaccharides

Neoglycolipid arrays involve labeling the carbohydrate by reductive amination. The primary amine and the C-1 aldehyde of the reducing sugar react to form a Schiff base, which is reduced to the mixed aryl/aliphatic secondary amine by sodium cyanoborohydride, as shown in Fig. 1. It is necessarily emphasized that the reaction must use excess monosaccharides in order to exhaust tetradecylamine.

The crude lipid-linked monosaccharides could be purified in a microtiter plate (Fig. 2). The method can been further extended to array complex carbohydrates.

Con A Assay

Figure 3 presents the structures of three monosaccharides, as well as fluorescence images and absorbance spectra of three neoglycolipids in microtiter plate are presented in Figure 3. It indicates that α-D-mannopyranoside, α-D-glucopyranoside, and α-D-galactopyranoside are epimers (Fig. 3A). The results show that mannose and glucose arrays both have fluorescence absorbance spectra but the galactose array does not (Fig. 3B,C). This proves that mannose and glucose can interact with *C. ensiformis* Con A but galactose cannot. The results are also in accord with the theoretical results. It demonstrates the C-1 hydroxys of mannose and glucose are not the main epitopes. Therefore, the method can be extended to study the steric hindrance effect of carbohydrate-protein interactions, namely the structure-function relations of carbohydrates.

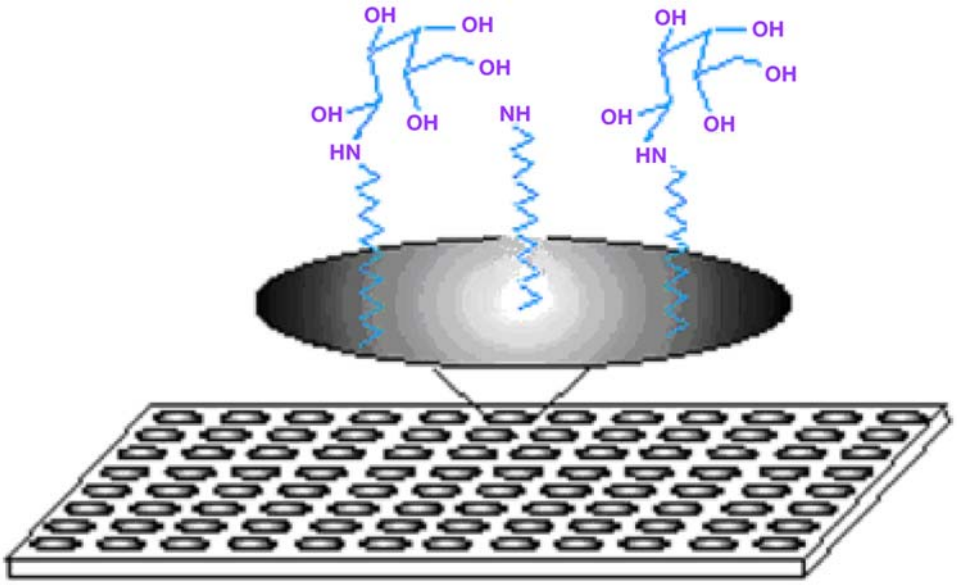

Fig. 2. Lipid-linked monosaccharides in microtiter plate well.

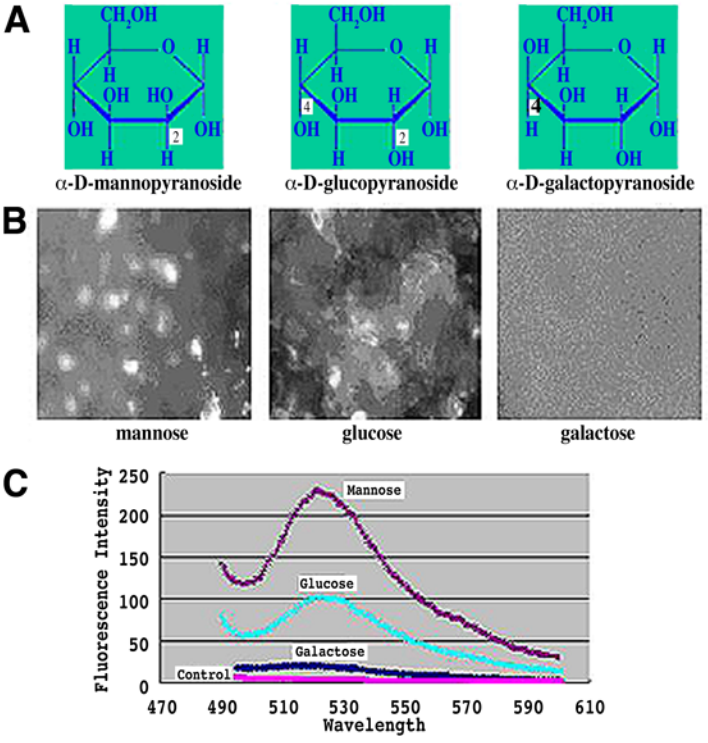

Fig. 3. **(A)** Structures of three monosaccharides; **(B)** fluorescence images of three neoglycolipids in microtiter plate; **(C)** absorbance spectra of three neoglycolipids in microtiter plate. Control denotes a tetradecylamine surface that lacked sugar.

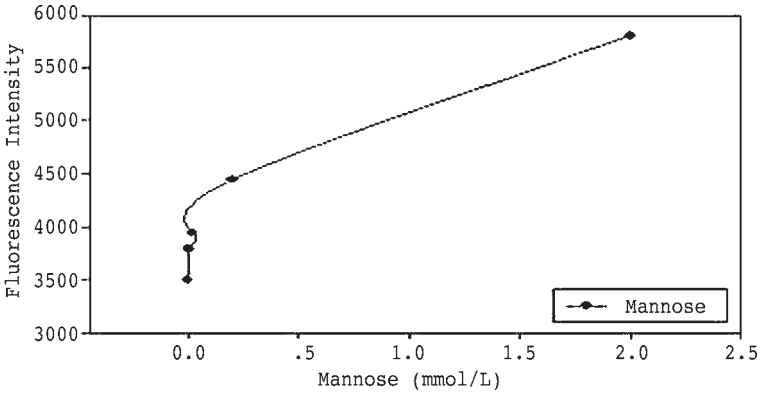

Fig. 4. Fluorescence analysis of FITC–Con A binding assay for reaction in microtiter plates at different concentrations (0.2 mmol/L to 2 mmol/L).

How Much Better Will Con A Bind to α-Mannose?

Figure 4 presents typical fluorescence analysis of FITC–Con A after subtracting the blank. It shows that the peak intensity increased with an increase in the concentration of α-mannose (0.2 mmol/L to 2 mmol/L).

Conclusion

In summary, this work shows an efficient methodology for the noncovalent assembly of saccharides in microtiter plates and in studying the structure–function relations of carbohydrates.

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