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Preparation and Properties of Dendritic sugar Immobilized Surface

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Novel saccharide sufaces with well-defined multivalency were prepared using glycodendrimer. The glycodendrimer α-mannose (α-Man) was synthesized and immobilized onto the acetylenyl- terminated gold substrate via click chemistry. The saccharide-immobilized substrates were evaluated with FTIR-RAS and XPS. The biological abilities of the saccharide-immobilized substrate were evaluated by quarts crystal microbalance (QCM) with the lectins of concanavalin A (ConA), and wheat germ agglutinin. The lectin binding to the glycodendrimer array was quantitatively analyzed, and the association constant (K_A) was revealed according to Scatchard plot. The kinetic constants were increase by glyco-dendrimer immobilization, and the multivalent effect of saccharide-lectin was exerted effectively.

Key words: Glyco-dendrimer, Self-Assembled Monolayer, Multivalent Effect

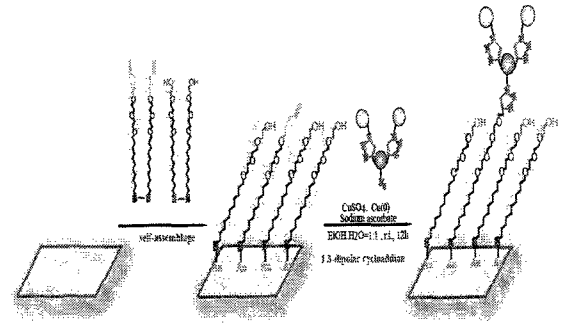
1. INTRODUCTION

Carbohydrate on cell surfaces is known as significant biological signal and plays important roles in various intercellular interaction.[1] Carbohydrate-protein interaction has specificity, but the affinity is usually weak. The carbohydrate-protein interaction in biological system is often amplified by the multivalency of carbohydrates, which is called the "multivalent effect" or "glyco-cluster effect".[2] These properties of saccharide, such as specific interaction and sensitive recognition had been paid much attention and applied to exploit various biomaterials and biodevices.[3] In particular, the biodevice called "sugar-chip" has been subject to the practical use but there is some problems, which the sensitivity is not high enough, and the multivalent effect is not used effectively Therefore, new technologies of overcoming the problems had been needed, and a development of more sensitive and quantitative device had been expected for practical use.

A method using self-assembled monolayer (SAM) is known to be effective manner to organize the surface which was served closely packed surface.[4] Combing with SAM, dense and closed surface with sugar ligands expected to be accomplished,[5] and led to exploit more sensitive devices.

On the other hand, accomplishing to adjust the multivalent effect and to exploiting the quantitative devices, it is necessary to construct the cluster which has a distinct rigid structure not like polymer. A concept of dendritic cluster was presented by Tomalia.[6] A structure of dendric cluster is in agreement with the purpose. Dendritic cluster has a distinct rigid structure, and is enabled to adjust the density of periphery with generation.

In this research, combining SAM with glyco-dendritic cluster (scheme1), we aimed to prepare more quantitative and sensitive sugar-surfaced device. After we prepared sugar derivatives immobilized substrate and characterized,subsequently the substrate was evaluated the recognition capability by the lectin binding assay with QCM.



Scheme1. Schematic illustration of the immobilization of saccharide.

2. EXPERIMENTAL

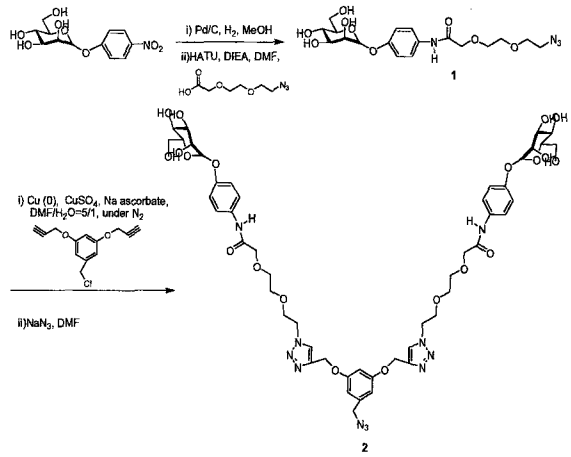
Materials

The following reagents were used as received: Palladium/charcoal (Pd/C) (Merck, NJ), *N*,*N*-dimethylformamide (DMF), sodium azide, sodium *L*-ascorbate, copper (II) sulfate pentahydrate, and methanol (Kanto Chemical, Tokyo, Japan), Concanavalin A (Con A) and Wheat germ agglutinin (WGA) (Seikagaku co., Tokyo, Japan). Phosphate buffered salts (+) and (-) (PBS (+) and (-)) as follow conventional method. *p*-Nitrophenyl pyranosides were prepared by conventional procedures. The acetylenyl-, and hydroxyl group terminated disulfides were synthesized according to the literature.[7]

Syntheses

The α-Man deriatives was synthesized as follows. The syntheses of the compounds were confirmed

by the following methods; ^{1}H (300 MHz) NMR spectra were recorded on a Varian Gemini 2000 equipped with a Sun workstation. The spectra were measured in DMF-d_7. Mass spectra were measured by MALDI-TOF-MS (Voyager, Applied Biosystems (Foster City, CA)) and ESI-MS (LCQ Deca xp, Thermo Fisher Scientific, MA).



Scheme 2. Syntheses of α-Man derivatives.

Syntheses of dendritic sugar

p-(*N*-(2-(2-Azide-ethoxy)ethoxy)amido)phenyl α-*D*-mannnoside (monomeric-α-Man (1)

*p*NP α-mannopyranoside (150mg, 0.498 mmol, 1eq) and Pd/C (15mg) was dissolved in MeOH (10mL) and were stirred under H_2 at room temperature. An hour later, we confirmed that the reaction was carried out. Pd/C was removed and the solution was evaporated to the brownish solid (136mg). Subsequently, the compound and [2-(2-Azide-ethoxy)ethoxy] acetate (189mg, 1.00mmol, 2.0eq) were dissolved in DMF (50mL), and stirred at 0°C. Then, HATU (176mg, 0.462mmol, 0.9eq) and N,N-diisopropylethylamine (120μl, 0.750 mmol, 1.5eq) were added to the solution. 10 hours later, we confirmed the reaction by rev.TLC (MeOH:H_2O=1:2) and the solution was evaporated to the crude product. The crude product was purified by rev. chromatography (H_2O:MeOH =2:1). At last, the white powder was isolated (214mg, 97%).

^{1}H-NMR (300MHz, DMF-d_7, ppm): δ9.50 (1H, s, -NHCO-), 7.66 (2H, d, *J*= 9.0 Hz, ArH, meta to α-Man), 7.11 (2H, d, *J*=9.0 Hz, ArH, ortho to α-Man), 5.41 (1H, s, H-1), 5.1 (1H, d, *J*=4.2, H-2), 4.94 (1H, d, *J*=5.1 Hz, H-$6_{pro\text{-}R}$), 4.72 (1H, d, *J*= 6.3 Hz, H-3), 4.51 (1H, t, *J*=5.8 Hz, H-5), 4.12 (2H, s, -NHCO$\underline{CH_2}$O-), 3.96 (1H, br, H-$6_{pro\text{-}S}$), 3.81 (1H, m, H-4), 3.73 (4H, m, -O$\underline{CH_2CH_2}$O$\underline{CH_2}$-CH_2-N_3), 3.67 (4H, m, -OCH_2CH_2O$\underline{CH_2CH_2}$-N_3). ESI-MS [positive]:465 $[M+Na]^+$.

Dendritic-α-Man (2)

1 (503 mg, 1.135mmol, 2.5eq), and 3,5-Bis (propagyloxy)benzyl chloride (106 mg, 0.454 mmol, 1eq) were dissolved in DMF. Sodium L-ascorbate (53.0mg, 0.270mmol, 0.6eq) and Copper sulfate (20.0mg, 0.126 mmol, 0.3eq) were dissolved in distilled water, and were added to the DMF solution. A few copper (0) was added in the solution, and stirred under nitrogen at room temperature for a day. The reaction was confirmed by TLC (reversed phase, H_2O:MeOH=1:1). The copper residue was removed by centrifugation, and water was evaporated. The crude compound was dissolved in DMF, and NaN_3 (125mg, 1.92 mmol, 4.5eq) was added. After the stirring reaction mixture at 70°C for a day, the solution was evaporated and purified by chromatography (reversed phase: H_2O: MeOH=3:1). The white powder was yielded (330mg, 63%).

^{1}H-NMR (300MHz, DMF-d_7, ppm): δ9.50 (2H, s, -NHCO-), 8.33 (2H, s, ArH, cyclic-triazole), 7.64 (4H, d, *J*= 9.0, ArH, meta to α-Man), 7.08 (4H, d, *J*=9.0 Hz, ArH, ortho to α-Man), 6.79 (1H, t, *J*=2.1 Hz, ArH, para to benzylazide), 6.70 (2H, br, ArH, ortho to benzylazide), 5.40 (2H, s, H-1), 5.20 (4H, s, -cyclic-triazole-C$\underline{H_2}$O- Ar-), 5.08 (2H, d, *J*=4.2 Hz, H-2), 4.94 (2H, d, *J*=5.4 Hz, H-$6_{pro\text{-}R}$), 4.74 (2H, d, *J*= 4.2 Hz, H-3), 4.67 (4H, t, *J*=5.1 Hz, -CH_2O $CH_2C\underline{H_2}$-cyclic-triazole), 4.50 (2H, t, *J*=6.0 Hz, H-5), 4.41 (2H, s, Ar-C$\underline{H_2}$-N_3), 4.08 (4H, s, -NHCOC$\underline{H_2}$O-), 3.96 (4H+2H, m, -CH_2OC$\underline{H_2}CH_2$-cyclic-triazole + H-$6_{pro\text{-}S}$), 3.81 (2H, m, H-4), 3.69 (8H, m, - O$\underline{CH_2}$ $\underline{CH_2}OCH_2CH_2$-cyclic-triazole).

MALDI-ToF-MS[positive]: 1148 $[M+Na]^+$.

Preparation of sugar-SAMs.

Acetylenyl-functionalized layers were formed on the substrate surface by SAM formation. The hydroxyl- and acetylenyl- terminated disulfides were dissolved at the molar ratio of hydroxyl-:acetylenyl- = 5:1 (total 20 mM) in a mixed solvent of ethanol and H_2O (EtOH:H_2O =2:1) (v/v). The gold substrate was immersed in the solution for 12 h, and then the substrate was rinsed with EtOH several times. Thereafter, the azide-terminated saccharides (**1** and **2**) were dissolved in the solution of EtOH:H_2O=1:2 (v/v) at 10 mM respectively, and the Cu(I) containing solution for the click reaction was prepared by a mixture of $CuSO_{4aq}$ (2mM) 4mL and Na ascorbate$_{aq}$ (5mM) 4mL with a small amount of Cu(0) powder.[8] The premixed Cu(I) solution was added to the solution of azide-terminated saccharides, and then the acetylenyl-terminated gold substrate was immersed in the mixed solution. The substrate was incubated for 12 h, and rinsed vigorously with EtOH and H_2O (Scheme 3).

Characterizations.

The SAM formation was analyzed as follows. The surface functional groups were analyzed by FTIR-reflection-absorption spectroscopy (RAS) (FTIR-4200 (JASCO, Co., Tokyo, Japan) equipped with a RAS attachment (Reflector 2, Harrick Scientific, NY)), and X-Ray photoelectron spectroscopy (XPS) (ESCA 5600 (ULVAC-PHI, Inc., Kanagawa, Japan)). In the SAM experiments, gold coated glass substrate s from Moritex (Tokyo, Japan) were used. The gold substrates for SAM

deposition were prepared by the exposition of O_3, and rinsed with EtOH.

Lectin recognition abilities

The saccharide-lectin interaction was analyzed using QCM (QCM-D, Q-Sense, Sweden). Each lectin were diluted to from 10^{-7}mM to 10^{-6}mM with adequate buffer (Con A to PBS+, WGA and BSA to PBS-). The changes of frequency by incubation of the solution were measured for 10 minutes, and the amount of immobilized lectin was calculated of the eventual frequency change by Sauerbrey equation.

3. RESULTS and DISCUSSION

Characterization of the substrate with saccharides

SAM formation was also measured by FTIR-reflection absorption (RAS) spectroscopy, and X-ray photoelectron spectroscopy (XPS). According to FTIR-RAS, vibrations around 3200-3400 cm^{-1} were observed in the reactive SAM layer corresponding to the hydroxyl groups of the SAM (Figure 1).

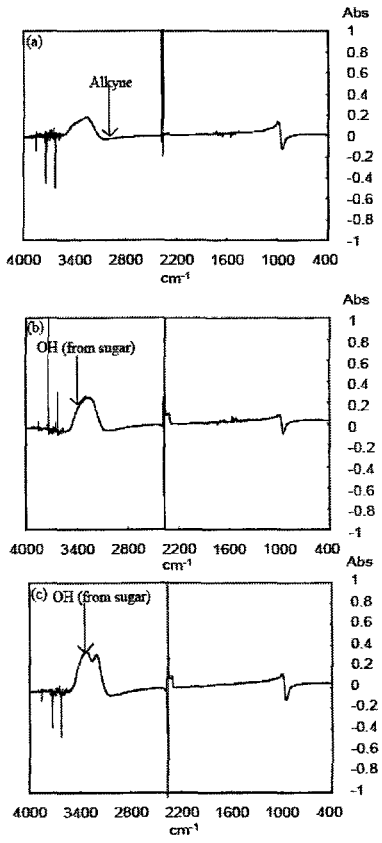


Figure 1. The FTIR-RAS spectra of the substrates;(a) SAM, (b) monomeric, and (c) dendritic. The changes by dendritic sugar immobilization was indicated by the allows, suggesting the density change of the sugar group.

In particular, as for the **1** and **2** immobilized layer, another peak which derived from the hydroxyl groups of sugar were observed at around .3350 cm^{-1}, and the peak intensity of G1(**2**) was larger than monomeric (**1**). Subsequently, with the XPS-spectra, the C1s region was analyzed by the pseudo-Voigt function. In the C1s region, the main peak observed for the bare gold substrate was observed around 283.8 eV and was assigned to the C-C bond. The SAM formed (**1**+**2**) substrate showed a shoulder peak around 284.1 eV due to the terminal C-O-C (ether) and C-OH groups. The observation of C-O-C peaks in the C1s region suggested saccharide immobilization (Figure 2).

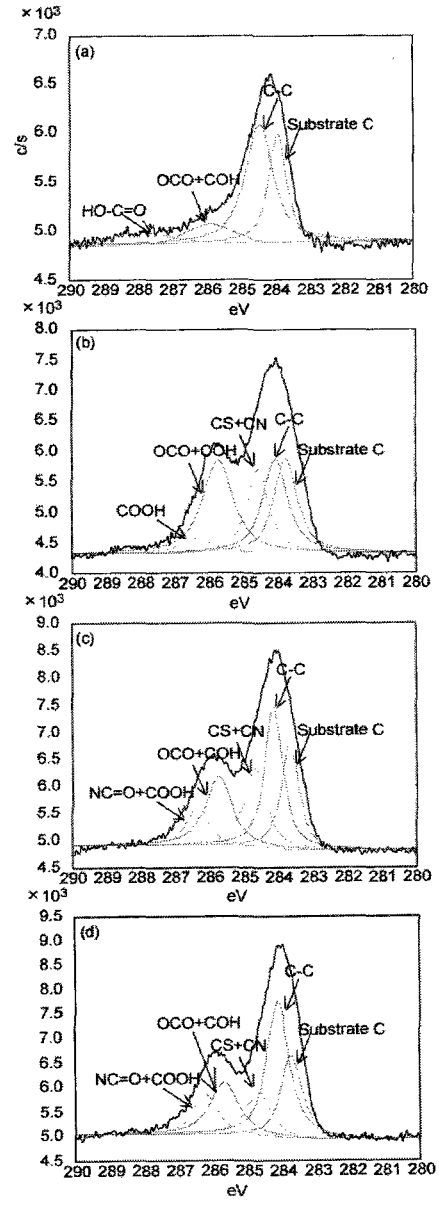


Figure 2. The XPS spectra of the substrates; (a) gold (b) SAM, (c) monomeric, and (d) dendritic. The changes by sugar-dendrimer immobilization was indicated by the allows, suggesting the density change of the sugar group.

Lectin recognition abilities.

First, we confirmed the binding specificity of dendritic a-Man immobilized substrate. The huge frequency change was observed with Con A, meaning that α-Man immobilized substrate recognized Con A selectively (Figure3). In addition, the frequency change of the substrate with WGA and BSA was much smaller.

Secondly, the amplification of interaction was evaluated compared of dendritic α-Man substrate with monomeric α-Man substrate. The frequency change of dendritic α-Man substrate to ConA solution at 0.2 μM was 3 times larger than that of the monomeric α-Man substrate. Subsequently, the kinetic constants were evaluated on the assumption of the Langmuir adsorption. According to Scatchard plot, the association constant (K_A) of the dendritic α-Man with ConA was 8.70×10^7 (M^{-1}), which is 1.5 times larger than that of monomeric α-Man (5.94×10^6 (M^{-1})). The increase of the interaction indicated the exhibition of the multivalent effect of dendritic saccharide SAM.

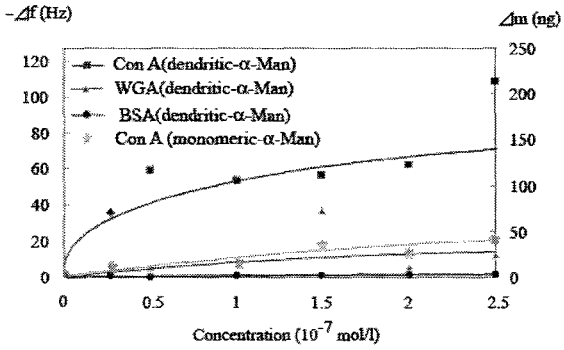


Figure 3. The recognition changes of the various α-Man derivatives immobilized substrate with QCM.

4. CONCLUSION

The glyco-dendritic cluster was synthesized via a click chemistry and by the divergent methods. Since the glyco-derivatives with terminal azide group was immobilized by the click reaction onto the acetylenyl-terminated self-assembled monolayer 、 the saccharide surfaces with well-defined multivalency were prepared. The interaction with lectin was specific and amplified by the multivalency of the glycol-dendritic cluster.

5. ACKNOWLEDGEMENTS

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