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Synthesis of Glycodendrimer via Click Chemistry and Protein Affinities

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Glyco-dendrimers carrying sulfate saccharides were efficiently synthesized via a cycloaddition of click chemistry. The interactions with a lectin of wheat germ agglutinin were evaluated by fluorescence spectroscopy. The interactions with amyloid β peptide were evaluated by CD spectra, thioflavin T assays and AFM observation. The interaction with the lectin and amyloid β peptide were affected by the size of the dendrimer and the kind of saccharide.

Key Words: Glyco-dendrimer, Click Chemistry, Amyloid β peptide.

1. INTRODUCTION

Cell surface saccharides play important roles in the living processes such as cell adhesion, pathogen infection and development of diseases.[1] These biological phenomena are related to the interaction between saccharides and proteins, which are usually weak, but are amplified by multivalency of so-called "glyco-cluster effect". In the living system, there are several saccharide assembliles such as raft and caveolae of glycolipid and the dendritic structure of glycoprotein. Artificial glyco-cluster compounds such as glyco-peptides, glyco-calixarenes, glyco-micelles, and glycopolymers have also been reported. Among the artificial glyco-clusters, the glyco-dendrimer has had a lot of attention due to the defined nano-structure and the large glyco-cluster effect.

In spite of the importance of the glyco-dendrimers, the syntheses are usually tedious due to the treatment of protective groups of sugar chemistry. Further, although there have been many reports of glyco-dendrimers so far, most were prepared by a simple modification of commercially available dendrimer. For the further understanding of the glyco-dendrimer molecular design, the selective and specific reaction is essential. For example, Staudinger ligation, Diels-Alder reaction, and cycloaddition between alkyne and azide can realize the facile preparation of various glycodendrimers. In particular, the click reaction (cycloaddition between alkyne and azide) has been utilized in the syntheses of various bioactive compounds, including the sugar derivatives.

In the present study, we investigated the syntheses of glyco-dendrimers using click chemistry, and their biological properties. We selected the sulfate saccharide of *N*-acetyl 6-sulfo-β-D-glucosaminide for dendrimer modification, which is one of the most abundant sulfate sugars in glycosaminoglycan.[2] We have recently synthesized glyco-polymer carrying 6-sulfo-β-D-GlcNAc, which interacted with Alzheimer amyloid β,[3] and prion protein.[4] In those studies, the polyamide derivatives of the sulfate saccharide were prepared, and thus, it was difficult to control the nano-structure and size of the glyco-cluster. Since the glycodendrimers have a defined nanostructure, they are useful to clarify the detailed protein-saccharide interaction and mechanism of the Alzheimer amyloid aggregation.

2. EXPERIMENTAL SECTION

Materials.

The following reagents were used as received: amyloid β(1-42) (Aβ((1-42))(Bachem AG, Switzerland), *p*-nitrophenyl *N*-acetyl β-D-glucosamine, Wheat germ agglutinin (WGA)(Seikagaku Co., Japan), propargyl bromide, dihydroxyl benzoic acid, diisopropyletylamine, dimethyl 5-hydroxy benzoic acid, *N*, *N*-dimethylformamide (DMF), $LiAlH_4$, Pd/C, thionyl chloride, thioflavin T (ThT), trimesic acid (Kanto Chemical, Japan), 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramet hyluronium hexafluorophosphate (HATU) (Peptide Institute, Japan), [2-(2-Chloroethox)ethoxy]ethanol, 5-hexynoic acid (TCI, Japan), 2,2,6,6-tetramethylpiperidinyl-1-oxy free radical (TEMPO) (Sigma-Aldrich, USA), and 1,3,5-triethylbenzene(ABCR Product, Germany).

CD Spectra

CD spectra for Aβ in a phosphate buffer solution were measured using a JASCO J-730 spectrometer with an optical cell of 1 mm path-length.

Thioflavin T (ThT) Fluorescence Assay

Amyloid fibril formation was evaluated by fluorescence emission of ThT using a JASCO FP-6500 spectrofluorophotometer and a 3-mm light-path quartz cuvette. After incubation at 37 °C, 5 μl of Aβ solution in buffer was added to 300 μl of ThT solution (50 μM) in the same buffer. After 10 s, the fluorescence intensity was measured at an excitation wavelength of 450 nm and an emission wavelength of 482 nm. The fluorescence intensity without Aβ was subtracted from

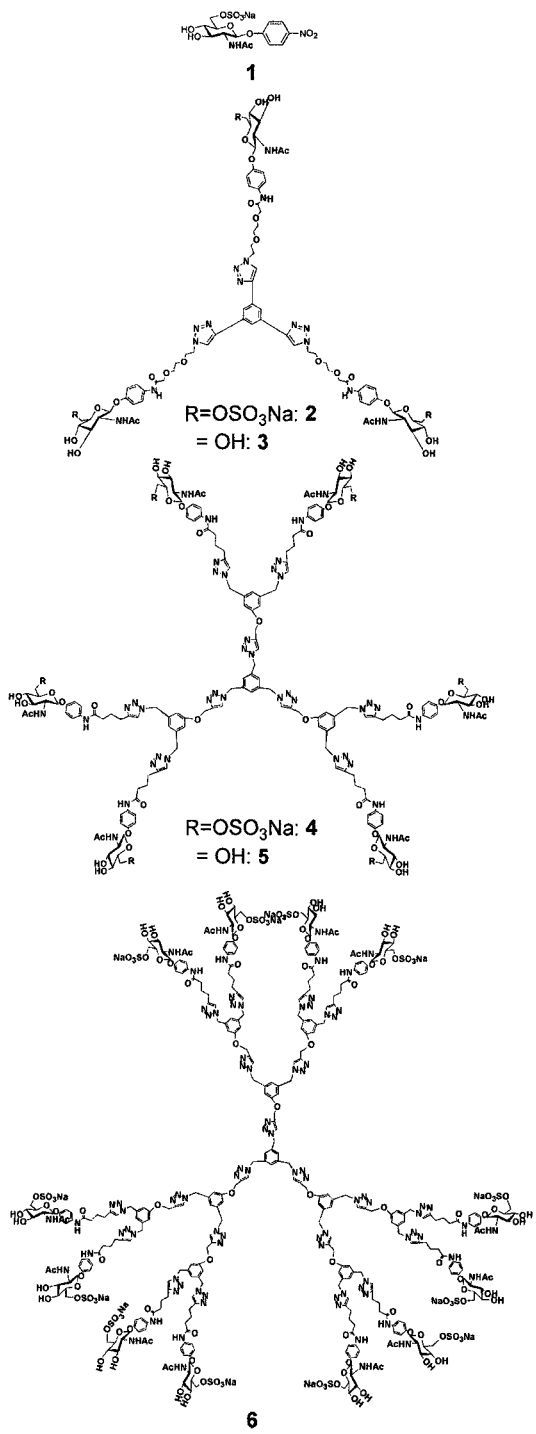

Figure 1 Glyco-dendrimers used in this manuscript.

that with Aβ and saccharide. The fluorescence intensity was taken as the average of at least four samples.

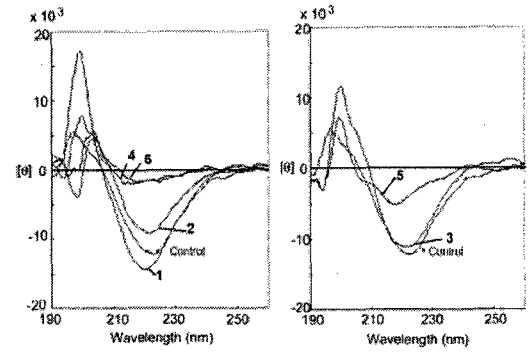

Figure 2 CD spectra for Aβ(1-42) in the presence of (a) sulphate GlcNAc derivatives (control, **1**, **2**, **4** and **6**), and (b) GlcNAc derivatives (**3** and **5**).

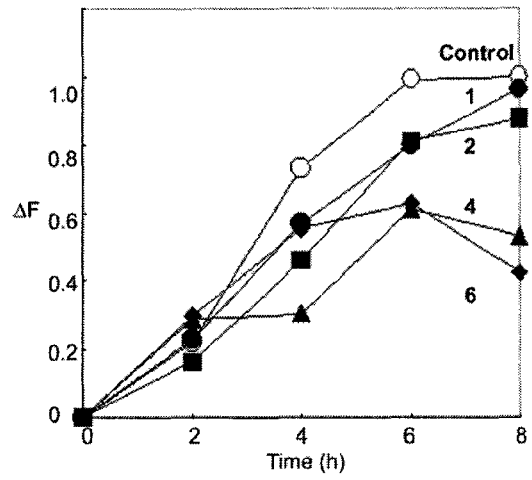

Figure 3 Time-course of the fluorescence change in ThT with Aβ(1-42) and sugar additives. (a) Effect of glycodendrimers; control (○), **1**(●), **2**(■), **4**(▲), and **6**(◆). The fluorescence of ThT was standardized by that of control sample after 8 h incubation.

AFM Measuments

AFM experiments were performed using a SPI-4000 atomic force microscope (Seiko Instruments Inc., Chiba Japan) equipped with a calibrated 20 μm xy-scan and 10 μm z-scan range PZT scanner. Measurements were performed in tapping mode. Aβ peptide was incubated in phosphate buffer (20 mM phosphate buffer 100 mM NaCl) at 37°C. The concentrations and incubation time of the peptide solution were 20 μM for 12 h (Aβ(1-42)). After incubation, 5 μl of each sample was placed on freshly cleaved mica and dried. The mica substrates were, then, washed with 100 μl of water.

***In vitro* Amyloid Formation of Aβ(1-42)**

Aβ(1-42) was dissolved in a 0.02 % ammonia

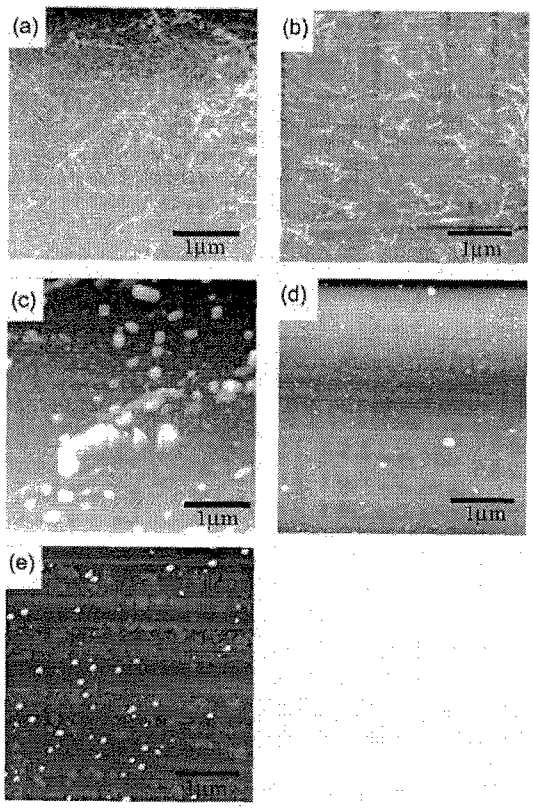

Figure 4 AFM observations of Aβ(1-42) (a) without additive (Control), (b) in the presence of **1**, (c) glycopolymer **2**, (d) **4** and (e) **6**.

solution at a concentration of 200 μM, and any aggregates formed were removed by centrifugation using a CS 120 FX (Hitachi, Tokyo, Japan) at 20,000 g for 30 min at 4°C. Next, the supernatant was mixed with phosphate buffer (20 mM phosphate buffer, pH 7.4, and 100 mM NaCl) to a final peptide concentration of 20 μM. The peptide solution was incubated with each glycopolymer at 37 °C. The sugar concentration of glycopolymers was 200 μM.

3. RESULT AND DISCUSSION

Syntheses of Glyco-dendrimer.

All of the glycodendrimers were synthesized by a click reaction followed by halogenation and azide substitution (Figure 1). The dendritic core without saccharide was synthesized by the selective coupling of click reaction with convergent method. The dendrimer of **3** and **4** were synthesized by the click reaction with alkyne-terminated 6-sulfo-β-D-GlcNAc to azido-modified dendrimer. A dendrimer **1** was synthesized by the click reaction of azido-terminated 6-sulfo-β-D-GlcNAc to 1,3,5-triethynylbenzene. The click reactions were conducted in the presence of Cu(I) generated *in situ* from $CuSO_4$ and sodium ascorbic acid. All the compounds were synthesized without protective groups due to the selective coupling. The dendrtic cores were water insoluble in aqueous solution, but the glyco-dendrimers with saccharide were readily soluble in aqueous solution due to the external hydrophilic saccharide.

Inhibition of Alzheimer Aggregation.

The inhibitory effect of the glycodendrimer on Aβ aggregation was investigated. First, the conformation of Aβ with glycodendrimer was evaluated by CD spectra (Figure 2). Aβ(42) showed a CD spectra with a minimum cotton effect around 220 nm, indicative of b-sheet structure. The addition of **1** showed a little increase of cotton effect. On the other hand, the addition of sulfate glycodendrimers decreased the minimum cotton effect around 220 nm, suggesting the inhibition of Aβ aggregation. The glycodendrimer with sulfate GlcNAc (**4** and **6**) remarkably inhibited the b-sheet conformation, indicative of the importance of multivalency. The glycodendrimer without sulfate group (**3** and **5**) also inhibited the β-sheet conformation, but the inhibitory effect of the glycopolymer on the conformation change was smaller than that of the glycodendrimer with sulfate GlcNAc, which means that the sulfate GlcNAc is essential in the inhibitory effect.

Then, the inhibitory effect of the dendrimer on Aβ aggregation was evaluated by the fluorescence change of ThT (Figure 3). The monomeric sulfate sugar (**1**) didn't show a change in the inhibitory effect. On the other hand, the dendrimers showed a remarkable decrease of ThT fluorescence due to the glyco-cluster effect. In particular, the denrimers **4** and **6** were effective on the amyloid inhibition. Those results were corresponding to CD spectra.

The morphology of Aβ was investigated with the addition of glycodendrimers (Figure 4) by AFM. Aβ(1-42) formed amyloid fibrils of 15-50 nm in width, and a few micrometers in length. The addition of a monomeric sulfate saccharide (**1**) didn't induce much difference in the amyloid, or rather induced the amyloid formation. The addition of glyco-dendrimer changed the morphology of Aβ aggregation. The dendrimer **2** induced the aggregation on the substrate. The size of the aggregates was 80-1300 nm in diameter, and the shapes of the aggregates were rectangular. The dendrimer **4** totally changed the morphology of the amyloid fibrils, and only the round objects with diameter from 10-200 nm were observed. In the case of dendrimer, **6** the morphology of the objects was changed in the round shape aggregates with a diameter from 50-150 nm. These AFM observations indicated that the glyco-dendrime with sulfate GlcNAc effectively inhibited the amyloid formation.

4. CONCLUSION

We synthesized glyco-dendrimer with *N*-acetyl 6-sulfo-β-D-glcosaminide via a click reaction without protective groups. The glycodendrimers interacted with GlcNAc recognition lectin and Alzheimer amyloid β. The affinities of the glycodendrimer with lectin were amplified by multivalent effect of dendrimer and dependent on the dendrimer generation. The glyco-dendrimer interacted with amyloid β effectively based on the glyco-cluster effect, and the aggregation of amyloid β was inhibited by the glyco-dendrimer carrying suflate GlcNAc.

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