Inhibition of Alzheimer Amyloid Aggregation with Sulfated Glycopolymers

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Glycopolymers carrying sulfated saccharides with modest sugar contents (11% and 28%) were found to suppress the formation of amyloid fibrils by amyloid β peptides (Aβ(1−42), Aβ(1−40), and Aβ(25−35)), as evaluated by thioflavin T assays and atomic force microscopy observation. Circular dichroism spectra showed that the conformation of amyloid β peptides depended on the glycopolymer additives, and that the glycopolymer additives reduced the β-sheet contents. Neutralization activity was confirmed by in vitro assay with HeLa cells. The sulfate group and the appropriate sugar contents were essential for the inhibitory effect.

Introduction

Alzheimer disease (AD) is characterized neuropathologically by extracellular deposition of amyloid senile plaques and neurofibrillary tangles in vulnerable AD brain regions.[1] These plaques are primarily composed of fibrils of amyloid β (Aβ) peptide, a small peptide composed of 39−43 amino acids.[2] The most abundant forms are 40 and 42 amino acids in length.[3,4] This kind of peptide is cleaved from a large protein called amyloid precursor protein (APP) by a secretases enzymes.[2] Production of amyloid β-peptide is a normal occurrence, although its function remains unknown. However, in AD patients the peptide forms ordered fibrillar aggregates.[1] This process involves the secondary structure transition from disordered random structure to ordered β-sheet conformation.[5]

Recently, saccharides on the cell surface were reported to play important roles in the deposition of Aβ peptides. Galβ1-3GalNAcβ1-4Gal(3-2αNeuAc)β1-4Glcβ1-1′Cer (GM1) ganglioside was shown to interact with Aβ and accelerate the aggregation of Aβ both in vivo[6−8] and in vitro.[9,10] Glycosaminoglycans (GAGs) (heparin, heparan sulfate, keratin sulfate, chondroitin sulfate, etc.) were found to be correlated with Aβ plaque formation.[11−15] These studies indicated that saccharides on the cell surface mediate and stabilize amyloid fibril formation. In other words these saccharides can be key compounds for the regulation of Aβ.

The interaction between saccharides (gangliosides and glycosaminoglycans) and Aβ peptides should be analyzed to clarify Alzheimer disease. However, the protein-saccharide interaction is usually too weak to detect,[16] and the structures of natural saccharides are too complex to analyze. On the other hand, the protein−saccharide interaction can be amplified by the multivalency of saccharide, so-called glycocluster effect. Synthetic glycoclusters have been reported to amplify the carbohydrate signals similarly to the natural saccharide ligands. In particular, synthetic glycopolymers substituted with pendant saccharides have attracted a great attention as synthetic glycoclusters in spite of the simple structure. We have reported the multivalent saccharides with glycopolymers, which effectively interacted with lectins and pathogenic toxins due to the multivalency.[17] Therefore, the glycopolymer carrying the bioactive saccharide is a useful tool to analyze the saccharide effects on Alzheimer disease.[18]

We paid particular attention to the sulfated glycosaminoglycans (GAGs), which induce the deposition of Aβ plaques, and prepared glycopolymer carrying sulfated sugars. It has been reported that glycopolymers with sulfated sugars exhibited the mimicry of GAGs, and that the polymers bound to bFGF for cell cultivation.[19] We synthesized glycopolymers using sulfate sugar of *N*-acetyl 6-sulfo β-D-glucosamine that is a component sugar of glycosaminoglycans (Figure 1).[20,21] Glycopolymers with various levels of sugar content were synthesized, and their interaction with Aβ was analyzed using thioflavin T (ThT) binding assays, atomic force microscopy (AFM), and circular dichroism (CD).

Experimental Section

Materials. The following reagents were used as received: amyloid β (1−40) (Aβ(1−40)), amyloid β (1−42) (Aβ(1−42)), amyloid β (25−35) (Aβ(25−35)) (Bachem AG, Switzerland), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) (Invitrogen Co. GIBCO, US), acrylamide, acryloyl chloride (TCI, Japan), *p*-nitrophenyl *N*-acetyl β-D-glucosamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Dojindo, Japan) Wheat germ agglutinin (WGA) (Seikagaku, Japan), 2, 2′-azobis-(2-amidinopropane)dihydrochloride (AAPD, Wako, Japan), and thioflavin T (ThT, Kanto Chemical, Japan).

Characterizations. ^{1}H (500 MHz) NMR spectra were recorded on a Varian Inova 500 equipped with a Sun workstation, and the spectra were measured in D_2O. Fluorescence spectra were measured on a JASCO FP-6500 (Jasco, Tokyo, Japan) at 25 °C. Size exclusion chromatography (SEC) was conducted with JASCO 800 high-performance liquid chromatography instrument on Shodex B804+B805 columns with PBS as an eluent. Molecular weights were estimated using a pullulan standard. CD spectra of Aβ were measured on a JASCO J-730 spectrometer using optical cell of 1 mm path length.

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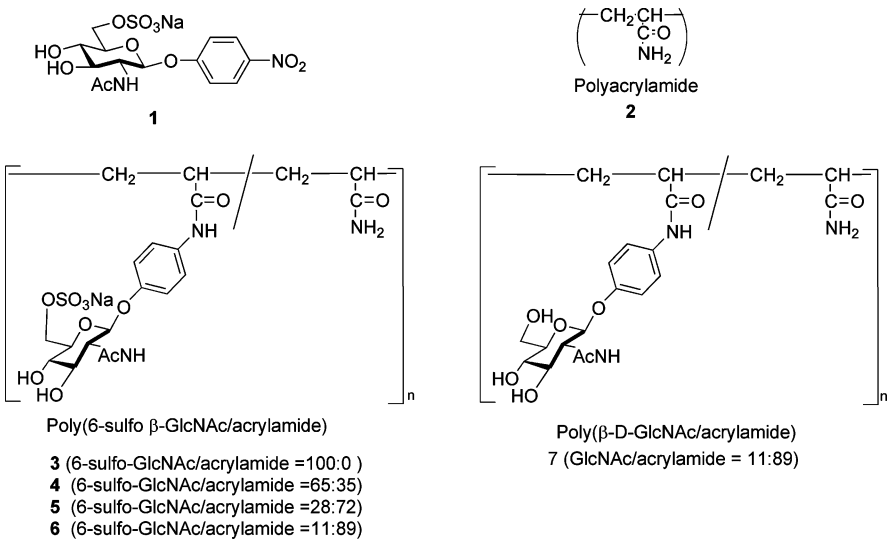

Figure 1. Molecular structures of the saccharide and glycopolymers used in this study.

Table 1. Copolymerization of *p*-(*N*-Acrylamido)phenyl Pyranoside with Acrylamide[a]

copolymer (sugar content)	monomer ratio **1**:acrylamide	time (h)	yield (%)	sugar unit in copolymer[b] (mol fr.)	$M_n \times 10^{-5}$ [c]	M_w/M_n
3 (100%)	1:0	15	65	1.0	6.6	1.3
4 (65%)	1:1	2.5	40	0.65	10	1.9
5 (28%)	1:4	1.5	71	0.28	3.4	1.8
6 (11%)	1:9	1	63	0.11	6.0	2.0

[a] Solvent (H_2O) 300 μL, AAPD 3 mol %, 60 °C. [b] Determined by ^{1}H NMR, [c]by pullulan standard.

Glycopolymers.[21] *p*-(*N*-Acrylamido)phenyl pyranosides were synthesized via hydrogenation of *p*-nitrophenyl pyanosides, and the subsequent treatment with acryloyl chloride. The *p*-(*N*-acrylamido)-phenyl pyranosides were copolymerized with acrylamide using 3 mol % of a radical initiator of AAPD (Figure 1). The resulting polymers were purified by dialysis in cellulose tubes with a molecular weight cutoff of 3500. The sugar content of those copolymers was estimated by ^{1}H NMR spectroscopy. The molecular weight and sugar contents of the polymers are summarized in Table 1. Polyacrylamide (**2**) ($M_n = 5.5 \times 10^5$, $M_w/M_n = 1.1$) and poly(*p*-acrylamidophenyl β-D-GlcNAc-*co*-acrylamide) (GlcNAc = *N*-acetyl-glucosaminide) (**3**) ($M_n = 3.0 \times 10^4$, $M_w/M_n = 2.7$) were also synthesized as references.

Lectin Recognition Assay. A lectin recognition assay was carried out by inhibition of lectin-induced hemagglutination as described in the literature.[22] The binding constants of glycopolymers were evaluated using a FITC-labeled WGA lectin, by Scatchard plots of fluorescence spectroscopy results.

Thioflavin T (ThT) Fluorescence Assay.[11] 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 that with Aβ and saccharide. The fluorescence intensity was measured as the average of at least four samples.

AFM Measuments.[23] AFM experiments were performed using 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 in tapping mode. Aβ peptides (1−40, 1−42, 25−35) were 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)), 50 μM for 24 h (Aβ(1−40)), and 100 μM for 24 h (Aβ(25−35)). After incubation, 5 μL of each sample was placed on freshly cleaved mica and dried. Then, the mica substrates were washed with 100 μL of water.

CD Spectra. CD spectra for Aβ in a phosphate buffer solution were obtained at 37 °C using a JASCO J-730 spectrometer with an optical cell of 1 mm path-length.

In Vitro Amyloid Formation of Aβ(1−42).[24] Aβ(1−42) was dissolved in 0.02% ammonia solution at a concentration of 200 μM, and any aggregates formed were removed by centrifugation using a CS 120 FX (Hitachi, Tokyo, Japan) at 16 000*g* at 4 °C for 3 h. 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.

In Vitro Amyloid Formation of Aβ(1−40).[24,25] Aβ(1−40) amyloid fibrils were prepared using the seed-dependent extension reaction. For the preparation seeds, Aβ(1−40) was dissolved in 0.02% ammonia solution at a concentration of 500 μM, The peptide solution was treated in an ultrasonic bath (UT-105S, Sharp, Tokyo, Japan) at 0 °C for 1 min. The sonicated solution was diluted to 50 μM in phosphate buffer (50 mM sodium phosphate buffer, 100 mM NaCl, pH 7.4). The sonicated solution was diluted to 50 mM in phosphate buffer (50 mM sodium phosphate buffer, 100 mM NaCl, pH 7.4), and incubated at 37 °C for 24 h. The preincubated solution was resuspended and treated as seeds. An unsonicated 50 μM Aβ(1−40) solution in a 0.02% ammonia solution was centrifuged at 16,000 g at 4 °C for 3 h. The seeding solution (20 μg/mL) was added to the Aβ(1−40) solution and incubated at 37 °C for 24 h.

In Vitro Amyloid Formation of Aβ(25−35).[25] Aβ(25−35) amyloid fibrils were prepared using the seed-dependent extension reaction. For the preparation of seed, Aβ(25−35) was dissolved in DMSO at 5 mM and diluted to 500 μm with MilliQ water. Aggregates were removed by centrifugation at 200,000 g at 4 °C for 3 h. The supernatant was diluted to 100 μM with phosphate buffer (100 mM NaCl 50 mM phosphate buffer), and the solution was incubated for 7 days at 37 °C. The preincubated solution was treated as seeds. The peptide solution

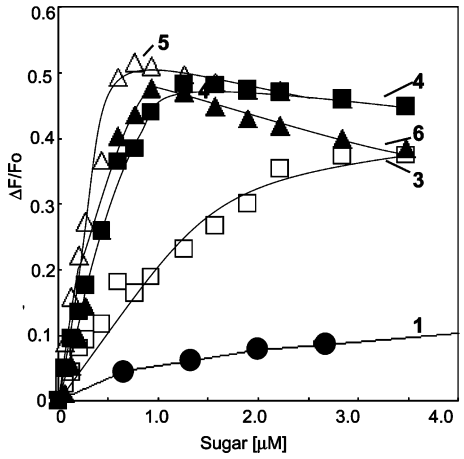

Figure 2. Fluorescence intensity changes of FITC-labeled wheat germ agglutinin (WGA) with varying sugar concentration. **1** (●), 3(100% sugar content; □), **4** (65% sugar content; ■), **5** (28%; △), and **6** (11%; ▲).

Table 2. Estimation of Lectin (WGA) Recognition Abilities

sample (sugar content)	affinity constant (M^{-1})[a]	MIC (M)[b]
1	7.6×10^5	n.d.[c]
3 (100%)	1.8×10^6	5.5×10^{-7}
4 (65%)	3.8×10^6	2.7×10^{-7}
5 (28%)	1.0×10^7	8.5×10^{-9}
6 (11%)	8.0×10^6	1.4×10^{-7}

[a] The affinity constants are based on the molarity of the glycosyl unit. [b] Minimum inhibitory concentration for inhibition of hemagglutinative activity. [c] n.d. means "not detectable".

was diluted to 100 μM with phosphate buffer (100 mM NaCl 50 mM phosphate buffer), and the seeding solution (5 μg/mL) was added to the peptide solution. The peptide solution was incubated at 37 °C for 24 h.

Neutralization of Aβ(1−42) with a Glycopolymer.[26] Neutralization activity of a glycopolymer (**6**) was examined in vitro using a HeLa cell cytotoxicity assay. HeLa cells were cultured in DMEM containing 10% FBS at 37 °C incubation with 5% CO_2. An aliquot containing 1×10^3 cells was transferred to each well of a 96-well plate with collagen coating (Collagen Type I-coated) (IWAKI, Tokyo, Japan), and DMEM was removed. Aβ(1−42) (60 μM) and glycopolymer (**6**) (600 μM) was incubated in HEPES buffer (20 mM HEPES, 100 mM NaCl) at 37 °C for 8 h in advance. The mixture of Aβ(1−42) and glycopolymer (**6**) in DMEM were added, where the final concentration of Aβ(1−42) and glycopolymer (**6**) was 20 μM and 200 μM, respectively. The mixture of the cells and the sample were incubated at 37 °C for 20 h. Then, supernatant was removed, and the cell indicator MTT−PBS (5 μg/mL, 10 μL) was added to the plate, and the cell was washed with 80% isopropanol and 0.04 N HCl (100 μL). The cell survival rate was calculated by absorbance at 570 and 650 nm. Results were calculated from four different wells.

Results

Interaction of Sulfated Glycopolymers with Lectin. Glycopolymers with various levels of sugar contents were synthesized by free radical polymerization (Table 1). The biological abilities of the glycopolymers were evaluated by the lectin recognition. The interaction of glycopolymers with the β-GlcNAc recognition lectin of WGA was estimated by the fluorescence spectroscopy of FITC-labeled WGA and by inhibition of a hemmaglutination assay (Figure 2, Table 2). The affinities for the lectin were in the order of **5** (sugar content: 28%) > **6** (11%) > **4** (65%) > **3** (100%) > **1** by both assays. The interaction of the glycopolymers with the lectin was much stronger than that of the monomeric saccharide (**1**). The glycopolymers with modest sugar content (**5** and **6**) showed approximately 3-fold stronger affinities than those with high sugar content (**3** and **4**).

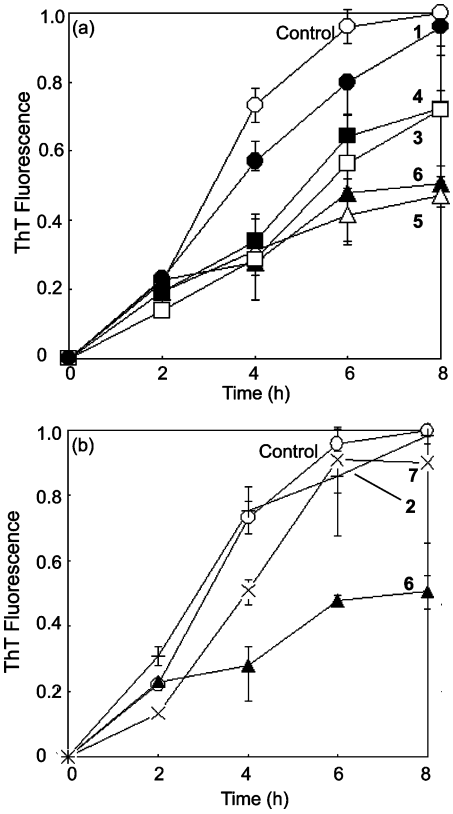

Figure 3. Time course of the fluorescence change in ThT with Aβ-(1−42) and sugar additives. (a) Effect of glycopolymers carrying sulfated sugars; control (○), **1** (●), **3** (100% sugar content; □, 4(65% sugar content; ■), **5** (28%; △), and **6** (11; ▲). (b) Effect of polymer additives without sulfated sugars; control (○), **2** (+), **6** (▲; reference), and **7** (X). The experiments were repeated at least four times. The fluorescence of ThT was standardized by that of control sample after 8 h incubation.

Thioflavin T (ThT) Fluorescence Assay of Aβ(1−42) Aggregation. Aβ(1−42) aggregation was inhibited by the addition of sulfated sugars and the inhibition was dependent on the kinds of sugars. The inhibitory effect of glycopolymers carrying sulfated sugar (**3**, **4**, **5**, and **6**) was much stronger than that of the monomeric sulfated sugar of *p*-nitrophenyl *N*-acetyl-6-sulfo β-D-glucosaminide (pNP-6-sulfo β-D-GlcNAc) (**1**), indicating a multivalent effect. In addition, the inhibitory effect was dependent on the sugar content of the glycopolymer. Glycopolymers with modest sugar contents, such as **5** (28%) and **6** (11%), showed a strong inhibition effect on Aβ(1−42) aggregation. Glycopolymers with high sugar contents, such as **3** (100%) and **4** (65%), exhibited only a weak inhibitory effect (Figure 3a). The degradation of the amyloid fibril was also examined by ThT assay, and the glycopolymer (**6**) was added to the solution of Aβ(1−42) after 8 h preincubation. The fluorescence of ThT was reduced about 30%, but amyloid didn't totally disappeared.

The aggregation of Aβ(1−42) was also examined by the addition of polyacrylamide (**2**) and glycopolymer with non-sulfated GlcNAc (**7**), but the inhibitory effect was not remarkable. Those data indicated that the sulfated group played an important role, and that the appropriate multivalent effect was necessary to inhibit Aβ(1−42) aggregation (Figure 3b).

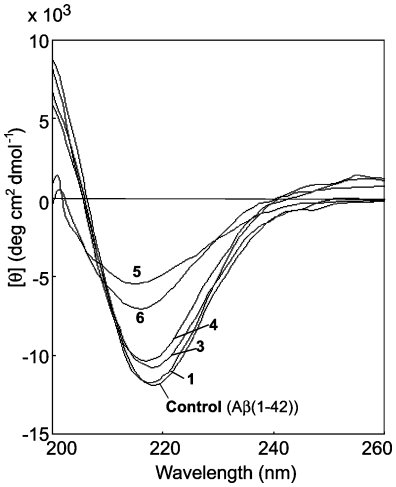

Figure 4. CD spectra for Aβ(1−42) in the presence of glycopolymers addtivies.

Conformation of Aβ(1−42) in the Presence of Glycopolymers.[5,27] Figure 4 shows CD spectra of Aβ(1−42) after 8 h incubation in the presence of saccharides. Aβ(1−42) has been reported to form a β-sheet-rich structure, as suggested by a CD minimum wavelength at 218 nm. The addition of glycopolymers decreased the Cotton effect of Aβ(1−42) and the minimum peak shifted from 218 nm to a shorter wavelength. The most remarkable change was attained with glycopolymer **5** (28% sugar content). The negative Cotton effect was decreased to 40%, and the negative peak shifted to 215 nm with **5**. The change in the CD spectra was consisted with the ThT assay for Aβ(1−42) aggregation. These results indicated that the addition of sulfated glycopolymers inhibited the conformational change of Aβ and thereby inhibited amyloid formation.

Morphology of Aβ(1−42) in the Presence of Glycopolymers. The morphology of Aβ(1−42) fibrils was observed by AFM (Figure 5). Aβ(1−42) readily formed amyloid fibrils of 15−50 nm in width, 5−15 nm in height and a few micrometers in length (Figure 5a). The addition of a monomeric saccharide, **1**, reduced the size of amyloid fibrils. The size of amyloid fibrils formed, when Aβ(1−42) was incubated with **1,** was 20−30 nm in width, about 10 nm in height and less than 1 μm in length, suggesting a weak inhibition of fibril formation (Figure 5b). The addition of polymeric saccharide (**3**) to Aβ(1−42) solution enlarged the fibril size with 60−300 nm in diameter, about 1 nm in height and 2−3 μm in length (Figure 5c). On the other hand, amyloid fibrils totally disappeared when Aβ(1−42) was incubated with **6** (Figure 5d). Aβ(1−42) formed spherical aggregates of 10−250 nm in diameter, and the size of these spherical objects was nonuniform. The morphology of Aβ(1−42) fibrils was consistent with the results of the ThT assay. The addition of sulfated saccharide changed the morphology of Aβ fibrils, and this glycopolymer efficiently reduced the formation of amyloid fibrils. The glycopolymer (**6**) formed spherical objects with 100−500 nm in diameter, which was similar to Aβ(1−42) with **6** (Figure 5e).

Inhibition of Amyloid Fibril Formation of Aβ(1−40). The formation of amyloid fibrils by Aβ(1−40) in the presence of saccharide was monitored by ThT assay and AFM analysis. We compared ThT fluorescence after 24 h incubation. In the presence of **6**, the ThT fluorescence of a solution of Aβ(1−40) was reduced to 32% of that without saccharide, suggesting inhibition of amyloid formation by Aβ(1−40) as well as Aβ-(1−42).

The morphology of Aβ(1−40) fibrils examined by AFM showed amyloid fibrils of 40−50 nm in width, about 10 nm in height and several micrometers in length (Figure 6a). The morphology of Aβ(1−40) in the presence of **1** was also fibrils, but the size of fibrils was a little smaller than that without saccharide (Figure 6b). The morphology of Aβ(1−40) with glycopolymer **3** (100% sugar content) was fibrils, and the size of fibrils was a little larger than those formed in the absence of

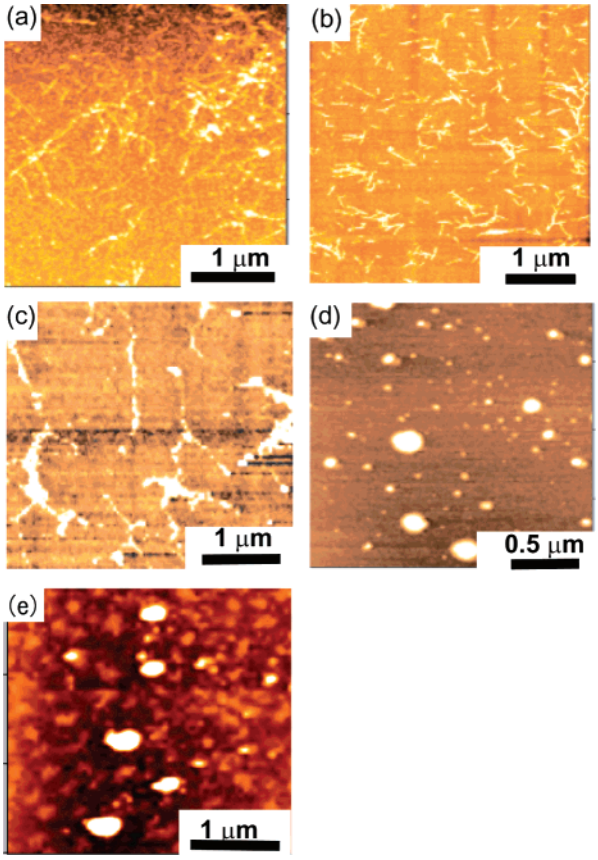

Figure 5. AFM observations of Aβ(1−42) (a) without glycopolymer, (b) in the presence of **1**, (c) in the presence of glycopolymer **3**, and (d) in the presence of glycopolymer **6**. The control sample of glycopolymer without Aβ(1−42) (e).

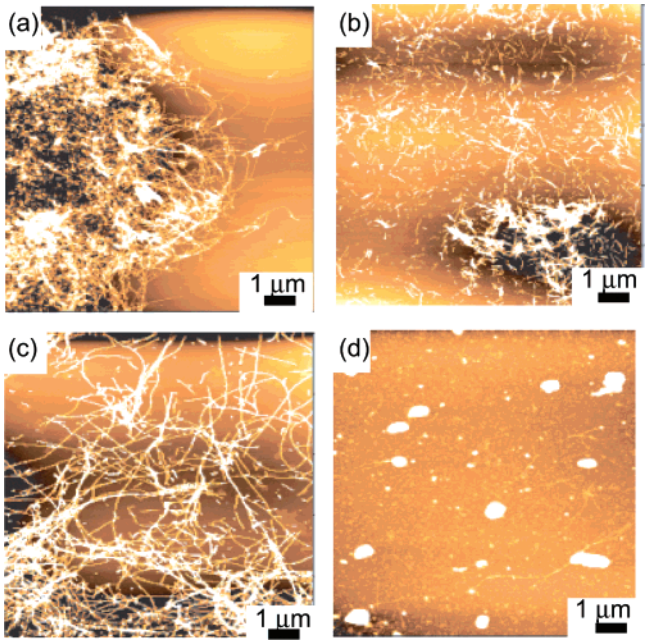

Figure 6. AFM observations of Aβ(1−40) (a) without glycopolymer, (b) in the presence of **1**, (c) in the presence of glycopolymer **3**, and (d) in the presence of glycopolymer **6**.

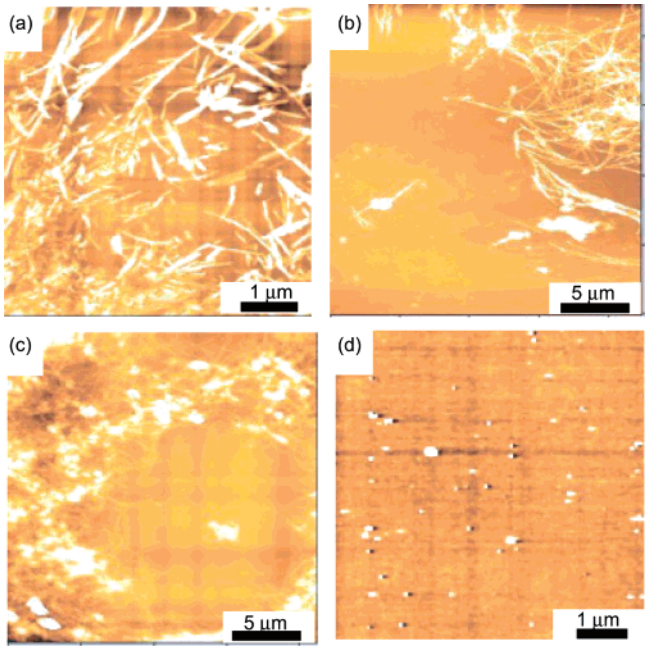

Figure 7. AFM observations of Aβ(25−35) (a) without glycopolymer, (b) in the presence of **1**, (c) in the presence of glycopolymer **3**, and (d) in the presence of glycopolymer **6**.

saccharide, being 40−70 nm in width, 7−10 nm in height and several micrometers in length (Figure 6c). Interestingly, when Aβ(1−40) was incubated with glycopolymer **6** (11%), the amyloid fibrils almost disappeared (Figure 6d).

Inhibition of Amyloid Fibril Formation on Aβ(25−35). The effect of saccharides was also analyzed using Aβ(25−35), which is known to be the cytotoxic fragment of Aβ peptide.[27] Amyloid formation by Aβ(25−35) was observed by AFM; fibrils were 10−60 nm in width, around 20 nm in height and several micrometers in length (Figure 7a). Amyloid formation was also observed in the presence of compounds **1** and **3** (Figure 7b,c). The diameter, the height, and the length of the fibrils in the presence of these compounds were around 50 nm, 20−40 nm, and several micrometers, respectively. On the other hand, the morphology of Aβ(25−35) with polymer **6** was totally different (Figure 7d). The amyloid fibrils totally disappeared and Aβ(25−35) formed round aggregates with 50−280 nm of diameter and around 20−40 nm of height.. The conformational change in Aβ(25−35) was also analyzed in the presence and absence of polymer. The CD spectra of Aβ (25−35) showed β-sheet like conformation with a negative Cotton effect around 220 nm. The negative Cotton effect was decreased by the addition of the glycopolymer **6** (Figure 8).

In vitro Neutralization of Aβ(1−42) with the Sulfated Glycopolymer. The sulfated glycopolymer was evaluated for its activity of neutralizing Aβ(1−42) to Hela cells (Figure 9). When the cells were incubated with Aβ(1−42) alone, Aβ(1−42) exhibited substantial cytotoxity reducing MTT activity by about 50%. However, co-incubation of Aβ(1−42) with a glycopolymer (**6**) showed a decrease in cytotoxity, indicating protection against Aβ(1−42). On the other hand, the incubation with the glycopolymer alone didn't show cytotoxity.

Discussion

It has been reported that the interaction between GAGs and amyloid β was associated with amyloid formation, and that the protein inclusion with GAGs acted as a scaffold to stimulate

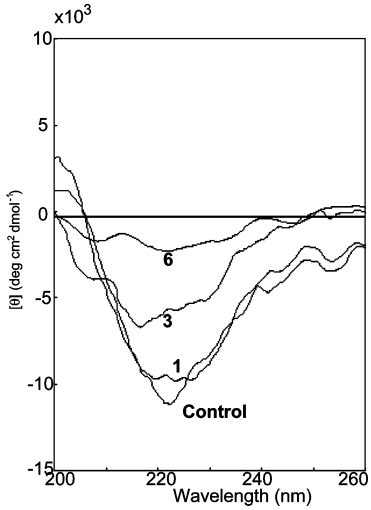

Figure 8. CD spectra for Aβ(25−35) in the presence of the glycopolymer additives.

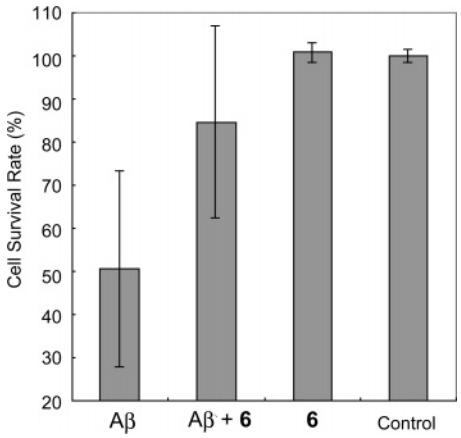

Figure 9. Neutralization of Aβ(1−42) with a glycopolymer (**6**). Survival rate of HeLa cells against Aβ(1−42) were followed in the presence of the glycopolymer. Results were the average of at least three samples.

the amyloidosis.[15] The interaction and the effect on amyloid formation were dependent on the kinds of GAGs, suggesting the conformation, the composed saccharide, and the physical properties affected the amyloid formation. In other words, the interaction with Aβ peptides was affected by saccharides, and can be controlled by the GAG mimics of glycopolymer. Therefore we examined the inhibitory effect of various glycopolymers.

In this investigation, the biological abilities of the glycopolymers, including the interaction with Aβ, were clearly dependent on their sugar contents due to the difference in polymer properties. The glycopolymer with modest sugar contents (**5** and **6**) bound to the proteins (Aβ and lectin) stronger than those with higher sugar contents (**3** and **4**). These results agreed that the appropriate multivalent compounds amplify the protein−sugar interaction, but that the inappropriate multivalent compounds didn't.[28] It has been reported that the protein-affinities of glycopolymers are much affected by their conformation and polymer flexibility.[28] For example, we have reported that glycopolymers with a rigid polymer backbone weakened protein-affinity.[29] Glycopolymers with higher sugar contents (**3** and **4**) might have a more rigid structure than that with lower sugar contents (**5** and **6**) due to the bulkiness of sugar side chain. The flexibile glycopolymers of **5** and **6** could easily access Aβ peptides and lectin. Since the morphology of glycopolymer (**6**)

was similar to that of Aβ peptides with glycopolymer, the round objects of Aβ with glycopolymer might be the inclusion complex of the glycopolymer with Aβ peptides. The inclusion of the glycopolymer with Aβ inhibited the interaction between Aβ peptides, which reduced the amyloid formation.

Glycopolymers with sulfated saccharide inhibited the amyloid fibril formation of all kinds of Aβ peptides (Aβ(1−42), Aβ-(1−40) and Aβ(25−25)). As glycopolymers without sulfated saccharide didn't show an inhibitory effect at all, the sulfated group was indispensable. Aβ(1−42) and Aβ(1−40) are rich in cationic residues (Arg15, His6, His13, His14, and Lys16), but Aβ(25−35) is not (only Lys28). Regardless of the net negative charges the sulfated saccharide group interacts not only through an electrostatic interaction, but also through hydrogen bonds with water on Aβ, the amide backbone, and the side chains of amino acids. Therefore, it is possible that glycopolymers with sulfated saccharides inhibit amyloid formation by many kinds of protein.[30]

When glycopolymers with sulfated sugars were added to peptide solutions, the morphology of fibrils changed from that of amyloid fibrils to round objects. Small round aggregates have been reported to show stronger cytotoxicity, but cytotoxicity of Aβ was reduced with glycopolymer due to the neutralization activity, suggesting glycopolymers are a promising inhibitor of Alzheimer amyloid[31,32]

Conclusion

The interactions between glycopolymers and Aβ peptides were analyzed in terms of Aβ aggregation. Glycopolymers carrying *N*-acetyl 6-sulfo-β-D-glucosaminide efficiently inhibited amyloid fibril formation. The appropriate multivalent effect was indispensable, similar to other sugar-protein interactions. The sulfated group in the saccharide was also essential for the inhibitory effect. The β-sheet rich structure was reduced, and the morphology of Aβ peptides changed from fibrils to round aggregates by the addition of sulfated glycopolymers. The cytotoxity of Aβ was neutralized by a sulfated glycopolymer. Sulfated glycopolymer was a novel inhibitor of Aβ peptides aggregation.

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Supporting Information Available. Degradation of amyloid fibril Aβ(1−42) using **6**, and ThT assay of Aβ(1−40). These materials are available free of charge via Internet at http://pubs.acs.org.

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