Controlled Synthesis of Amphiphilic Block Copolymers with Pendant *N*-Acetyl-D-glucosamine Residues by Living Cationic Polymerization and Their Interaction with WGA Lectin

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ABSTRACT: Amphiphilic block copolymers of vinyl ethers (VEs) having pendant *N*-acetyl-D-glucosamine (GlcNAc) residues were synthesized by living cationic polymerization of isobutyl VE (IBVE) and a VE carrying 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucose (**1**). Sequential block copolymerization of **1** and IBVE was conducted by the $CF_3COOH/EtAlCl_2$ initiating system in the presence of added base (1,4-dioxane) to yield the precursor block copolymer with a narrow molecular weight distribution ($\bar{M}_w/\bar{M}_n \sim 1.1$) and a regulated segment composition. Deprotection and subsequent N-acetylation of the precursor polymers afforded the GlcNAc-carrying amphiphilic block copolymers with a well-controlled structure. The interaction of the resultant GlcNAc-carrying block copolymer with wheat germ agglutinin (WGA) lectin, which is known to specifically recognize GlcNAc and its oligomers, was investigated. The association constants were evaluated by measuring the changes in fluorescence intensity of WGA observed upon addition of the GlcNAc-carrying polymer to an aqueous solution of WGA. It was clearly demonstrated that the GlcNAc-carrying polymers show a significant increase in binding affinity toward WGA lectin compared to GlcNAc and its oligomers.

Introduction

Recent progress in glycobiology has revealed that cell surface oligosaccharides play essential roles in various biological recognition processes, including intercellular recognition, adhesion, cell growth, and differentiation.[1] These recognition processes are essentially based on carbohydrate−protein interactions. However, it is known that individual interactions are generally of low affinity. In this respect, glycopolymers in which saccharide residues are incorporated to polymer backbones as pendant groups are emerging as important materials for the study on the carbohydrate−protein interactions.[2−9] When the density and relative spatial arrangements of the carbohydrate residues incorporated are appropriate, glycopolymers can induce marked enhancement of binding affinity toward proteins due to the multivalent recognition, which have been known as the "cluster effect".[2−8] Since a wide range of glycopolymers have proved to be very potent ligands against various lectins and viruses, studies on their synthesis and properties have become a growing research area.

In the area of polymer chemistry, amphiphilic block copolymers are known to exhibit various interesting properties such as surface activity, aggregate formation, and so on; therefore, some products are widely employed for technological applications.[10] Among a large variety of low-molecular-weight and macromolecular amphiphiles that exist in nature, many possess carbohydrate residues as hydrophilic components. Therefore, amphiphilic block copolymers with pendant carbohydrate residues can serve as mimics of naturally occurring amphiphiles such as glycolipids and glycoproteins for a better understanding of essential biological processes in living system. In addition, these artificial glycoconjugates can be applied in affinity chromatography, site-specific drug delivery systems, cell-culture systems, and so on. Despite the considerable progress in the development of synthetic strategies to prepare block copolymers of various architectures, by far less research has been directed to the synthesis of block copolymers with pendant saccharide residues owing to the synthetic difficulty.

Recently, new methodologies for the synthesis of glycopolymers with well-defined structure have been developed, utilizing cationic polymerization,[11] ring-opening polymerization of *N*-carboxyanhydrides,[12] ring-opening metathesis polymerization,[13,14] and, more recently, nitroxide-mediated free radical polymerization.[15] A key advantage of these systems is the "living" nature in the polymerization, which can lead to the formation of glycopolymers with controlled molecular weights and narrow polydispersities. Moreover, living polymerization techniques have the advantage of yielding well-defined block copolymers by the sequential addition of two or more monomers. In a previous work, we have succeeded in the synthesis of well-defined amphiphilic block copolymers with pendant glucose residues by living cationic polymerization of vinyl ethers (VEs), and demonstrated that thin films of these polymers exhibit various types of microphase-separated surface structures.[11b]

We herein describe the controlled synthesis of a novel amphiphilic block copolymer **4** with pendant *N*-acetyl-D-glucosamine (GlcNAc) residues by living cationic polymerization. Moreover, binding properties of the GlcNAc-substituted block copolymers with wheat germ agglutinin (WGA) lectin, which specifically recognizes GlcNAc and its *β*-(1,4)-linked oligomers, are also discussed.

Results and Discussion

1. Synthesis of Amphiphilic Block Copolymers with Pendant GlcNAc Residues. (a) Block Copolymerization. As shown in Scheme 1, precursor block

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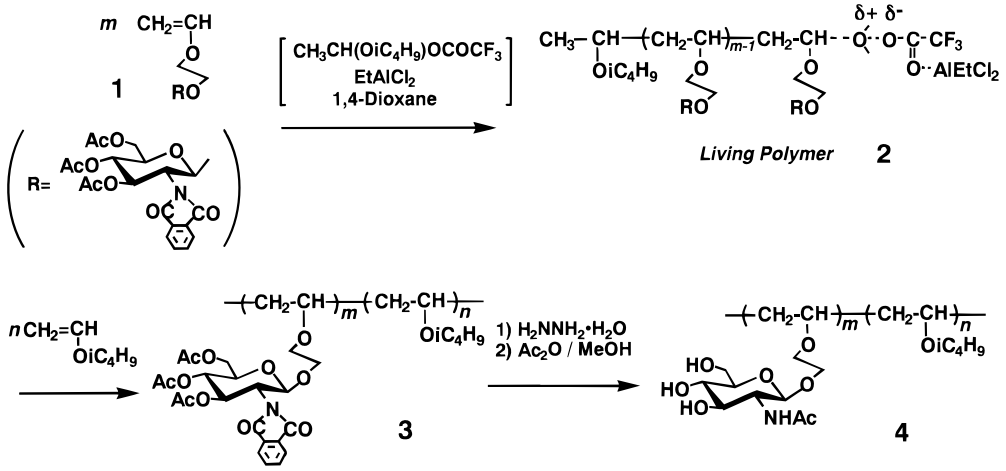
Scheme 1



copolymer **3** was synthesized by a sequential living cationic copolymerization of VE **1** with fully substituted glucosamine residues and isobutyl VE (IBVE), employing the trifluoroacetic acid/ethylaluminum dichloride ($CF_3COOH/EtAlCl_2$) initiating system[16] in the presence of added base (1,4-dioxane) in toluene at 0 °C. As an initiator was employed herein a trifluoroacetate, CH_3-$CH(OiBu)OCOCF_3$, which was prepared by addition of trifluoroacetic acid to IBVE, to avoid the side reaction in the initiation process.[11a] VE **1** has proved to undergo living cationic polymerization under these conditions.[11c]

Preliminary experiments showed that the polymerization sequence from **1** to IBVE is suitable for the quantitative formation of the narrow-polydispersity block copolymers with the well-controlled structure. The opposite polymerization sequence (from IBVE to **1**) indeed yielded the block copolymers but the blocking efficiency was below 100%. Thus, **1** was first polymerized to give a living poly(**1**) with a narrow molecular weight distribution (MWD), and the second-stage polymerization was subsequently carried out by the addition of IBVE.

Figure 1 shows the MWD curves of the prepared block copolymers **3** with varying the degree of polymerization ($\overline{DP_n}$) of the poly(IBVE) (PIBVE) segment and a constant $\overline{DP_n}$ of the poly(**1**) segment. As seen in the figure, increasing the amount of IBVE in the feed leads to an increase in the molecular weight of the resulting block copolymers while the MWD stays narrow. The overall results of the block copolymerization were summarized in Table 1. Thus, low-polydispersity block copolymers **3** with different molecular weight and composition (*m*/*n*) can be synthesized. Figure 2A shows the ^1H NMR spectrum of the block copolymer **3**. The spectrum exhibits the absorptions assigned to the poly(**1**) subchain (peaks a and b) together with those of the PIBVE subchain (peak c), supporting the formation of the block copolymer. $\overline{DP_n}$ for the poly(**1**) segment (*m*) was derived from the ^1H NMR spectrum of the first-stage homopolymer **2** quenched just before the addition of the second monomer; namely, *m* was calculated based on the peak intensity ratio of the anomeric proton of the glucopyranose ring (H-1) to the pendant methyl protons in the initiator residue at the α-end.[17] Similarly, the segment composition ratio (*m*/*n*) of the block copolymer **3** could be estimated from the ratio of the peak intensity of the anomeric proton of the glucopyranose ring (H-1) to that of the pendant methyl protons in the PIBVE segment (peak c). As shown in Table 1, *m*/*n* values evaluated by ^1H NMR spectroscopy were in good agreement with the calculated values.

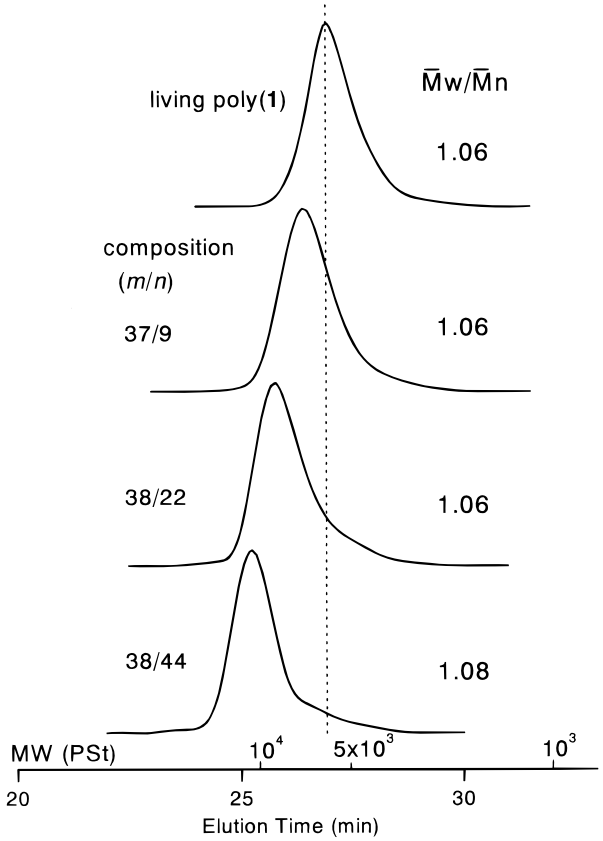


Figure 1. MWD curves of **1**−IBVE block copolymers and their precursors obtained by the sequential polymerization of **1** and IBVE in toluene at 0 °C: (A) starting living poly(**1**), $[\mathbf{1}]_0 = 0.20$ M, $[CH_3CH(OiBu)OCOCF_3]_0 = 5.0$ mM, $[EtAlCl_2]_0 = 10.0$ mM, $[1,4\text{-dioxane}]_0 = 0.6$ M; (B−D) block copolymers from sample A, where $[IBVE]_{add}$ = (B) 0.05, (C) 0.10, and (D) 0.20 M.

(b) Deprotection and N-Acetylation into GlcNAc-Carrying Block Copolymers. We have previously reported that the deprotection of poly(**1**) proceeded quantitatively to afford the water-soluble VE polymer with pendant glucosamine residues.[11c] The protecting groups of block copolymer **3** were similarly removed with employing hydrazine monohydrate, and then the resultant amino group at C-2 position was selectively acetylated with acetic anhydride in methanol[18] to afford

Table 1. Characterization of Homopolymers 2 and Block Copolymers 3

code	structure	$\overline{DP}_n$ (m/n)[a] calcd[b]	obsd[c]	$\bar{M}_n$[c]	$\bar{M}_w/\bar{M}_n$[d]
2a	homopolymers	13/0	13/0	6600	1.07
2b		20/0	20/0	10100	1.05
2c		40/0	36/0	17700	1.06
3a	block copolymers	20/5	20/8	10900	1.06
3b		20/20	20/22	12300	1.04
3c		20/100	20/108	20900	1.06
3d		40/10	37/9	19600	1.06
3e		40/20	38/22	21400	1.06
3f		40/40	38/44	23600	1.08

[a] $\overline{DP}_n$ shows the degree of polymerization of each segment. [b] $m = [\mathbf{1}]_0/[\text{initiator}]_0$; $m/n = [\mathbf{1}]_0/[\text{IBVE}]_0$. [c] Determined by ^{1}H NMR peak intensity ratio (see text). [d] Estimated by polystyrene-calibrated GPC.

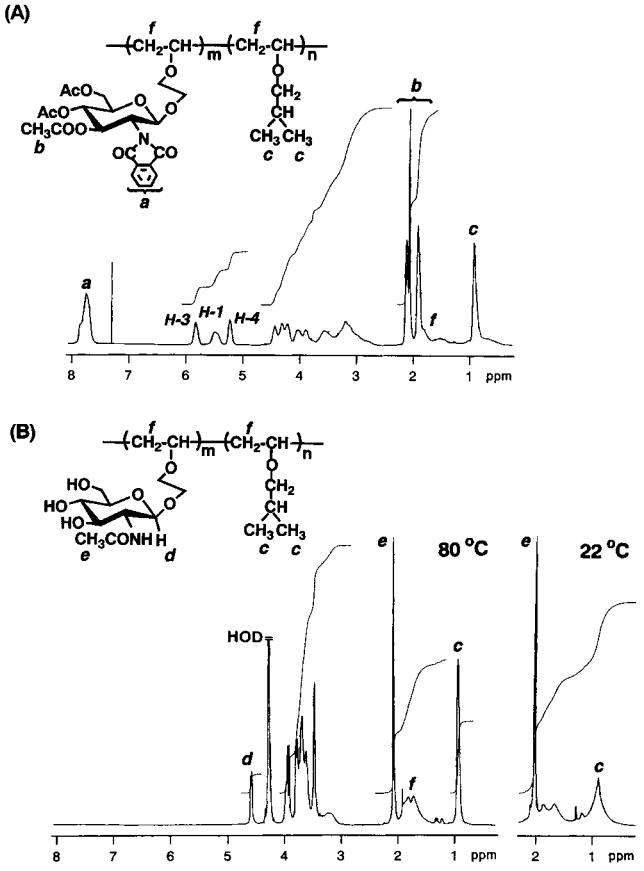


Figure 2. ^{1}H NMR spectra of **3** in $CDCl_3$ at room temperature (A) and of **4** in D_2O at 80 °C (B) and at 22 °C (B, inset): (A) precursor block copolymer **3** (m/n = 38/22); (B) deprotected product **4** obtained from sample A.

the target amphiphilic block copolymer **4** with GlcNAc residues. As shown in Figure 2, comparison of the ^{1}H NMR spectra of **3** and **4** revealed that the deprotection and selective *N*-acetylation proceeded without any side reactions. In Figure 2B, for example, the absorption of acetyl (peak b) and phthalimide group (peak a) entirely disappeared and the pyranose ring protons (H-3 and H-4) shifted upfield (3.3−3.8 ppm), while the peaks assignable to the anomeric proton (β-form) of the GlcNAc residue (peak d) and *N*-acetyl groups (peak e) newly appeared. In contrast, the signals of the PIBVE segments (peak c) and that of the main chain (peak f) remained unchanged, indicating the absence of undesirable side reactions during the reactions. Further evidence for the successful conversion into the GlcNAc-carrying counterpart was provided by ^{13}C NMR. For instance, five carbonyl signals due to the acetoxyl and phthalimide groups completely disappeared and a new peak originating from the acetamide group instead appeared after the reactions (see Experimental Section).

The precursor block copolymer **3** was soluble in organic solvents (toluene, chloroform, etc.), but insoluble in water. In contrast, **4** was found to be water-soluble except for **4c** with the longest hydrophobic PIBVE subchain. The spectrum of **4**, shown in Figure 2B, was taken in D_2O at 80 °C. A decrease of temperature to 22 °C brought about the broadening and decrease in intensity of the peak arising from the methyl protons in the PIBVE segment (see peak c, inset of Figure 2B), in sharp contrast to the peak assignable to the *N*-acetyl groups in poly(GlcNAcVE) (PGlcNAcVE) segment (peak e) being unchanged. In a previous paper, similar trends were observed for the glucose-carrying amphiphilic block copolymers of VEs. These spectral changes would indicate, though qualitatively, the formation of micellar aggregations with a core consisting of the hydrophobic PIBVE subchains, rendering the inner PIBVE block restricted in motion at decreased temperatures.[11b] Further studies on the micellization behaviors of the carbohydrate-substituted block copolymers have been made by NMR, GPC, and light-scattering measurements and will be reported elsewhere.[19]

2. Interaction of GlcNAc-Carrying Block Copolymer 4 with WGA Lectin. WGA is one of the well-studied plant lectins and specifically recognizes GlcNAc, its β-(1,4)-oligomers, and *N*-acetyl neuraminic acid.[20] The specificity and stoichiometry of GlcNAc-carrying ligands for WGA have been intensively studied using a variety of analytical techniques.[21] For instance, a fluorescence method was successfully applied to investigate the interactions of carbohydrate-binding lectins with glycopolymers, where clustering glycopolymers were shown to induce a much enhanced binding affinity compared to the corresponding mono- and oligosaccharides.[8] Here, our interest was focused on the analysis of binding affinity of GlcNAc-carrying VE polymers to WGA, in particular on how the block copolymer architecture affects on the GlcNAc residues-WGA interaction. The association constant (K_a) of GlcNAc-carrying polymer and WGA can be evaluated by measuring the changes in fluorescence intensity of tryptophan residues which locate near the binding site in WGA.[21a,b]

Upon excitation at 290 nm, WGA showed a spectrum with a maximum emission at 350 nm. As shown in Figure 3, when the lectin was saturated with GlcNAc-carrying block copolymer (**4f**, m/n = 38/44), the maximum fluorescence intensity was enhanced by 42% and the emission maximum was shifted to 340 nm. This behavior is characteristic of tryptophan residues whose environment has become less polar than the original one due to the binding of WGA with GlcNAc residues.[21a,b] Thus, the spectral change indicates that WGA specifically interacts with GlcNAc residues in the block copolymers.

Figure 4A shows a plot of $\Delta F/F_0$ vs [S], where ΔF is the change of fluorescence intensity at its maximum obtained in the presence of GlcNAc residues of a concentration [S] in **4**, and F_0 is the fluorescence intensity of WGA alone. The plot shown in Figure 4A exhibits a sigmoidal shape, which is known to be characteristic of a positive cooperative binding process.[22−24]

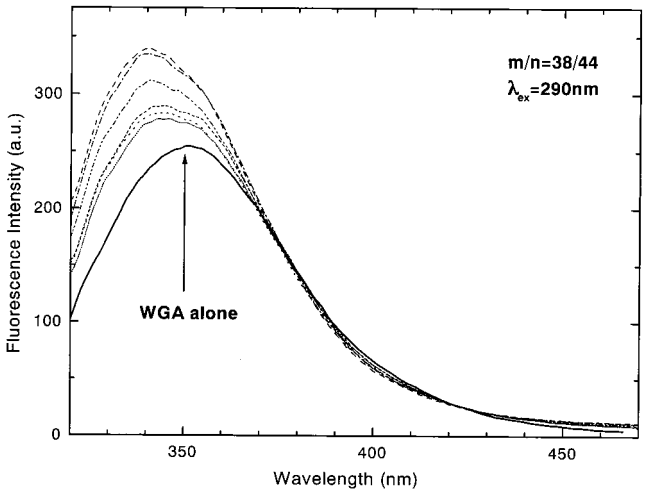


Figure 3. Fluorescence spectra of WGA (0.75 μM) and its complexes obtained by the addition of various amount of GlcNAc-carrying block copolymer **4** (m/n = 38/44) measured at 17 °C, in 50 mM HEPES buffer (pH 7.5) containing 0.3 M NaCl.

Replotting of this binding curve based on the Hill model gave a linear graph (Figure 4B) where the slope is defined as the "Hill coefficient" (h), which is the measure of cooperativity.[22] K_a was also evaluated from the intercept of this plot to give $K_a = 1.9 \times 10^5$ M^{-1} for **4f** (m/n = 38/44). Molecular characteristics, Hill coefficients, and K_a values of the GlcNAc-carrying homopolymers and block copolymers, as well as monomeric GlcNAc, are summarized in Table 2. K_a for GlcNAc was found to be 6.8×10^2 M^{-1}, being in good agreement with the literature value (6.9×10^2 M^{-1}).[21b] It is noteworthy that all the GlcNAc-carrying polymers displayed more than 10^2 times enhancement in recognition ability relative to monovalent GlcNAc itself. Moreover, the GlcNAc-carrying polymers exhibited a Hill coefficient of ca. 2 or above, while monomeric GlcNAc gave approximately unity,[21b] indicating the positive cooperativity in the present glycopolymer−WGA system. The enhanced binding affinity of the GlcNAc-containing polymers toward WGA would be attributed to the multivalent interaction, which has been well-documented for various types of glycopolymers. Such cooperative binding is one of the most interesting phenomena in both natural[23] and artificial[24] recognition systems and is relevant to a better-understanding of molecular recognition processes in living system.

Table 2. Interaction of GlcNAc-Carrying Polymer with WGA

code	structure	composition m/n ($\overline{DP_n}$)	MW[a]	K_a [M^{-1}][b]	Hill coefficient (slope, h)
	GlcNAc		221	6.8×10^2	1.1
	homopolymers	13/0	3800	4.4×10^4	1.9
		20/0	5800	5.9×10^4	2.3
		36/0	10500	8.0×10^4	2.5
4a	block copolymers	20/8	6600	3.3×10^5	2.3
4b		20/22	8000	1.6×10^5	2.2
4d		37/9	11700	3.5×10^5	2.2
4e		38/22	13300	2.9×10^5	2.5
4f		38/44	15500	1.9×10^5	2.4

[a] Theoretical values based on the degrees of polymerization. [b] Calculated from a Hill plot based on GlcNAc units in the polymers.

Figure 5 plots the K_a values of the block copolymers having a constant GlcNAc-carrying segment length (m = 38 or 20) as a function of the $\overline{DP_n}$ of the PIBVE segment, n. Apparently, all the block copolymers are superior to the homopolymers (n = 0) in binding affinity toward WGA. In addition, of particular interest was that, irrespective of the $\overline{DP_n}$ of the GlcNAc-carrying hydrophilic segment (m = 38 or 20), the incorporation of the hydrophobic PIBVE subchain of $n \cong 10$ (**4a** and **4d**) brings about the most effective binding with an increase in K_a by 1 order of magnitude relative to those of the homopolymers. The block copolymers with a

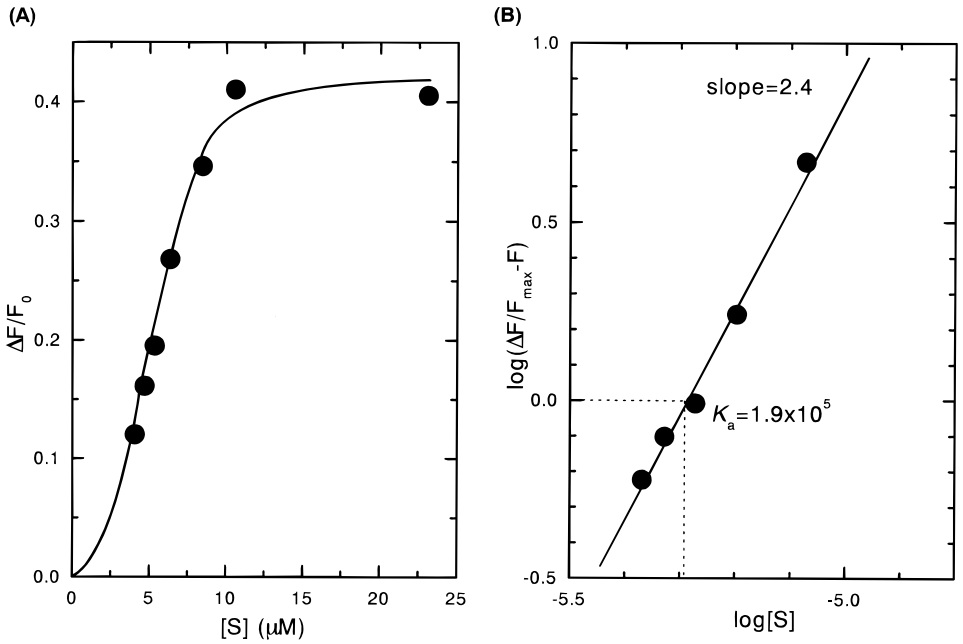


Figure 4. (A) Plot of $\Delta F/F_0$ against [S], where ΔF is the change of fluorescence intensity at its maximum, F_0 is the fluorescence intensity of WGA alone, and [S] is the concentration of GlcNAc-carrying polymer added (based on GlcNAc unit). (B) Hill plot for the data in Part A, where F_{max} is the fluorescence intensity of WGA saturated with ligands. See Figure 3 for the measurement conditions.

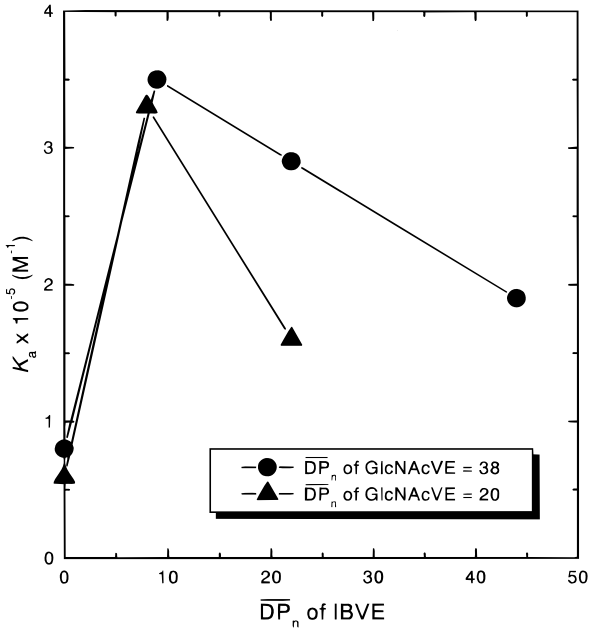


Figure 5. Effect of $\overline{DP}_n$ of PIBVE segment (n) on K_a of the binding of GlcNAc-carrying block copolymers with WGA. See Figure 3 for the measurement conditions.

PIBVE segment of $n > 10$ give rise to somewhat lower recognition ability (**4b**, **4e**, and **4f**). One possible explanation for the discrepancy among the block copolymers with different PIBVE lengths is that the conformation of the GlcNAc-carrying subchain may be somewhat changed in aqueous media, depending on the size of the hydrophobic segment, which in turn affects the relative spatial arrangements of the polymer-bound GlcNAc residues. Recalling that the GlcNAc-carrying block copolymers are capable of forming micellar aggregates in aqueous media as indicated by the NMR measurement, the efficient binding of these block copolymers toward WGA lectin may be related to their dissolution state in aqueous media. Here, a critical micelle concentration (CMC) of sample **4f** (m/n = 38/44) was evaluated by the fluorescence technique using pyrene as a fluorescent probe.[25] The observed CMC value was 6.5×10^{-7} M in block copolymer concentration, which corresponds to 25 μM based on the GlcNAc residues. This value is slightly higher than the concentration region examined for the binding study (see, Figure 4A). However, taking into account the difficulties with the accurate determination of the onset of the micellization, we cannot clearly describe the dissolution state of the block copolymers in the concentration range examined in the present study. The nature and mechanism of the binding characteristics of the GlcNAc-substituted block copolymers are under investigation and will be discussed elsewhere. It should be emphasized that glycopolymers with novel architecture are available by the synthetic methodology reported here and that the binding behavior of these block glycopolymers are closely related to their composition.

In conclusion, we have synthesized GlcNAc-carrying amphiphilic block copolymers with a well-defined structure by living cationic polymerization. It was demonstrated that the molecular weight and segment composition of the block copolymers can be controlled by regulating the feed molar ratio of the initiator to monomer and of the two monomers. The GlcNAc-carrying polymer showed a much increased recognition ability toward WGA lectin compared to monovalent GlcNAc itself and its β-1,4-linked oligomers.[26] Moreover, the GlcNAc-carrying block copolymers exceed the homopolymer in binding strength. Thus, the synthetic methodology in the present study would be beneficial for designing glycopolymer-based advanced materials.

Experimental Section

Materials. VE **1** was prepared as described previously.[11c] Commercial IBVE (Tokyo Kasei Kogyo Co., Ltd.) was washed with 10% aqueous sodium hydroxide and then with water, dried overnight over potassium hydroxide (pellets), and distilled twice over calcium hydride before use. Toluene and *n*-hexane were purified by the usual methods and distilled twice over calcium hydride prior to use. 1,4-Dioxane as an added base was distilled twice over sodium wire. $EtAlCl_2$ (Kanto Chemicals; 0.96 M solution in *n*-hexane) was used as received. IBVE−CF_3COOH adduct was synthesized as reported.[27] Wheat germ agglutinin (WGA) was purchased from Sigma.

Polymerization Procedures. Polymerization was carried out under dry nitrogen in a baked glass tube equipped with a three-way stopcock at 0 °C. The reaction was initiated by the sequential addition of chilled solutions of the initiator (IBVE−CF_3COOH adduct in toluene; 0.80 mL) and of $EtAlCl_2$ (in toluene; 0.40 mL) into the monomer solution (VE **1** in toluene; 6.40 mL) containing 1,4-dioxane (0.40 mL, 0.6 M) under dry nitrogen and was terminated with chilled ammonical methanol (5 mL). The quenched reaction mixture was washed with dilute hydrochloric acid and then with water to remove the initiator residues, evaporated to dryness under reduced pressure, and vacuum-dried to yield the product polymers. ^{13}C NMR ($CDCl_3$): δ 19.74 (CH_3 in IBVE), 20.59, 20.74, 20.88 (CH_3 in Ac), 29.01 (CH in IBVE), 38.0−41.5 (main chain $-CH_2-$), 54.56 (C-2), 61.97 (C-6), 66.0−68.0 ($-OCH_2CH_2O-$), 68.97, 70.78, 71.81 (C-3, C-4, C-5), 73.55 (main chain $-CH-$), 75.73 (CH_2 in IBVE), 98.36 (C-1), 123.42, 131.16, 134.17 (aromatic), 166.91, 167.50 (C=O in phth), 169.22, 169.80, and 170.33 (C=O in Ac).

Deprotection and N-Acetylation of Precursor Block Copolymers. To a solution of precursor block copolymer **3** (250 mg) dissolved in a 1,4-dioxane (8 mL)/methanol (4 mL) mixture was added hydrazine monohydrate (10 equiv for the protecting groups in the polymer). After being stirred at 60 °C for 4 h, the mixture was evaporated under reduced pressure to give a white residue which was dissolved in methanol (6 mL). Then acetic anhydride (1.2 mL) was added, and the mixture was stirred at room temperature for 1.5 h. After solvent removal, the residue was dissolved in water, filtered, and dialyzed against deionized water. Finally the solution was concentrated by evaporation and freeze-dried to afford the target amphiphilic block copolymer **4** (105 mg; yield, 65%). ^{13}C NMR (D_2O): δ 19.80 (CH_3 in IBVE), 22.81 (CH_3 in NHAc), 28.95 (CH in IBVE), 38.0−41.5 (main chain $-CH_2-$), 55.67 (C-2), 61.00 (C-6), 66.5−68.5 ($-OCH_2CH_2O-$), 70.08 (C-4), 73.5−75.0 (C-3, main chain $-CH-$), 75.5−77.0 (C-5, CH_2 in IBVE), 100.98 (C-1), and 173.84 (C=O in NHAc).

Polymer Characterization. Size exclusion chromatography (SEC) was carried out in tetrahydrofuran (THF) at 40 °C on a TOSOH HLC-8020 high-speed liquid chromatograph equipped with polystyrene gel columns (Tosoh G2500H, G3000H, and G4000H) con-

nected to refractive index/ultraviolet dual detectors. The number-average molecular weight ($\bar{M}_n$) and polydispersity ratio ($\bar{M}_w/\bar{M}_n$) were estimated on the basis of a polystyrene calibration. NMR spectra were obtained with a JEOL AL-400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Fluorescence Measurements. Fluorescence spectra of WGA were recorded on a Perkin-Elmer LS-50B luminescence spectrometer with excitation at 290 nm. The solutions were contained in 10-mm quartz cells maintained at 17 °C. The concentration of WGA in 4-(2-hydroxyethyl)-1-piperazineethanesulfononic acid (HEPES) buffer (50 mM, containing 0.3 M NaCl, pH 7.5) was estimated to be 0.75 μM so that the absorbance at 280 nm was below 0.1 ($\epsilon = 1.5$ cm^2/mg).[28]

CMC of the GlcNAc-containing block polymer was evaluated under the conditions identical with those employed for the K_a determination, except for the absence of WGA lectin. 10-mm quartz cell was filled with ca. 3 mL of the sample solution with [pyrene] = 5.0×10^{-7} M. The sample solutions were prepared as reported.[25] The solutions were sealed and stirred for 1 day to allow the micelles to be equilibrated with pyrene. Excitation spectra were recorded at 17 °C with $\lambda_{em} =$ 390 nm.

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References and Notes

(1) For recent reviews, see: (a) Kobata, A. *Eur. J. Biochem.* **1992**, *209*, 483. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683.

(2) For reviews, see: (a)*Neoglycoconjugates: Preparation and Applications*; Lee, Y. C., Lee, R. T., Eds.; Academic Press: San Diego, CA, 1994. (b) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321. (c) Bovin, N. V.; Gabius, H.-J. *Chem. Soc. Rev.* **1995**, *24*, 413. (d) Kiessling, L. L.; Pohl, N. L. *Chem. Biol.* **1996**, *3*, 71. (e) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 692.

(3) (a) Kobayashi, K.; Sumitomo, H.; Kobayashi, A.; Akaike, T. *J. Macromol. Sci., Chem.* **1988**, *A25*, 655. (b) Kobayashi, K.; Tsuchida, A.; Usui, T.; Akaike, T. *Macromolecules* **1997**, *30*, 2016.

(4) Roy, R.; Tropper, F. D. *J. Chem. Soc., Chem. Commun.* **1988**, 1058.

(5) (a) Manning, D. D.; Hu, X.; Beck, P.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 3161. (b) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 9931.

(6) Rathi, R. C.; Kopecková, P.; Kopecek, J. *Macromol. Chem. Phys.* **1997**, *198*, 1165.

(7) (a) Choi, S.-K.; Mammen, M.; Whitesides, G. M. *Chem. Biol.* **1996**, *3*, 97. (b) Sigal, G. B.; Mammen, M.; Dahmann, G.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 3789.

(8) (a) Nishimura, S.-I.; Furuike, T.; Matsuoka, K.; Maruyama, K.; Nagata, K.; Kurita, K.; Nishi, N.; Tokura, S. *Macromolecules* **1994**, *27*, 4876. (b) Nagata, K.; Furuike, T.; Nishimura, S.-I. *J. Biochem.* **1995**, *118*, 278. (c) Furuike, T.; Nishi, N.; Tokura, S.; Nishimura, S.-I. *Macromolecules* **1995**, *28*, 7241.

(9) Wulff, G.; Schmid, J.; Venhoff, T. *Macromol. Chem. Phys.* **1996**, *197*, 259 and references therein.

(10) (a) Halperin, A.; Tirell, M.; Lodge, T. P. *Adv. Polym. Sci.* **1992**, *100*, 31. (b) Laschewsky, A. *Adv. Polym. Sci.* **1995**, *124*, 1.

(11) (a) Minoda, M.; Yamaoka, K.; Yamada, K.; Takaragi, A.; Miyamoto, T. *Macromol. Symp.* **1995**, *99*, 169. (b) Yamada, K.; Yamaoka, K.; Minoda, M.; Miyamoto, T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 255. (c) Yamada, K.; Minoda, M.; Miyamoto, T. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 751.

(12) (a) Aoi, K.; Tsutsumiuchi, K.; Okada, M. *Macromolecules* **1994**, *27*, 875. (b) Tsutsumiuchi, K.; Aoi, K.; Okada, M. *Macromol. Rapid. Commun.* **1995**, *16*, 749.

(13) Fraser, C.; Grubbs, R. H. *Macromolecules* **1995**, *28*, 7248.

(14) Nomura, K.; Schrock, R. R. *Macromolecules* **1996**, *29*, 540.

(15) Ohno, K.; Tsujii, Y.; Miyamoto, T.; Fukuda, T.; Goto, M.; Kobayashi, K.; Akaike, T. *Macromolecules* **1998**, *31*, 1064.

(16) Kishimoto, Y.; Aoshima, S.; Higashimura, T. *Macromolecules* **1989**, *22*, 3877.

(17) The $CF_3COOCH(CH_3)O\boldsymbol{X}/EtAlCl_2$/1,4-dioxane initiating system employed in the present study can induce a well-defined living polymerization of VEs under adequate conditions.[16] Thus, the resultant polymers invariably possess one initiator residue (*X*) per chain as the α-end group. In this case, the use of $CF_3COOCH(CH_3)OCH_2CH(CH_3)_2$ brings about the formation of the polymers with the isobutoxy group at the α-end.

(18) Durette, P. L.; Meitzner, E. P.; Shen, T. Y. *Tetrahedron Lett.* **1979**, 4013.

(19) Yamada, K.; et al. To be published.

(20) (a) Allen, A.; Neuberger, A.; Sharon, N. *Biochem. J.* **1973**, *131*, 155. (b) Bhavanandan, V. P.; Katlic, A. W. *J. Biol. Chem.* **1979**, *254*, 4000.

(21) (a) Lotan, R.; Sharon, N. *Biochem. Biophys. Res. Commun.* **1973**, *55*, 1340. (b) Privat, J.-P.; Delmotte, F.; Mialonier, G.; Bouchard, P.; Monsigny, M. *Eur. J. Biochem.* **1974**, *47*, 5. (c) Nagata, Y.; Burger, M. M. *J. Biol. Chem.* **1974**, *249*, 3166. (d) Jordan, F.; Bassett, E.; Redwood, W. R. *Biochem. Biophys. Res. Commun.* **1977**, *75*, 1015. (e) Banis, G.; Lee, R. T.; Lee, Y. C.; Freire, E. *Biochemistry* **1992**, *31*, 12624. (f) Wright, C. S.; *J. Biol. Chem.* **1992**, *267*, 14345.

(22) Connors, K. A. *Binding Constants*; John Wiley: New York, 1987.

(23) (a) Newton, A. C.; Koshland, D. E., Jr. *J. Biol. Chem.* **1989**, *264*, 14909. (b) Williams, B. A.; Chervenak, M. C.; Toone, E. J. *J. Biol. Chem.* **1992**, *267*, 22907. (c) Sharman, G. J.; Searle, M. S.; Benhamu, B.; Groves, P.; Williams, D. H. *Angew. Chem., Int. Ed. Eng.* **1995**, *34*, 1483. (d) Chervenak, M. C.; Toone, E. J. *Biochemstry* **1995**, *34*, 5685.

(24) (a) Tabushi, I. *Pure Appl. Chem.* **1988**, *60*, 581. (b) Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F.-T. *J. Am. Chem. Soc.* **1990**, *112*, 3860. (c) Kikuchi, Y.; Tanaka, Y.; Sutarto, S.; Kobayashi, K.; Toi, H.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10302. (d) Takeuchi, M.; Imada, T.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2096.

(25) (a) Wilhelm, M.; Zhao, C.-L.; Wang, Y.; Xu, R.; Winnik, M. A.; Mura, J.-L.; Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033. (b) Astafieva, I.; Zhong, X. F.; Eisenberg, A. *Macromolecules* **1993**, *26*, 7339.

(26) In ref 21b, K_a values for $(GlcNAc)_2$ and $(GlcNAc)_3$ were estimated to be 4.5×10^3 and 2.0×10^4, respectively.

(27) Kamigaito, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1991**, *24*, 3988.

(28) Privat, J.-P.; Delmotte, F.; Monsigny, M. *FEBS Lett.* **1974**, *46*, 224.

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