Synthesis of well-defined cyclic glycopolymers and the relationship between their physical properties and their interaction with lectins

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出版情報：Polymer Chemistry. 13 (38), pp.5453-5457, 2022-09-10. Royal Society of Chemistry
バージョン：
権利関係：

COMMUNICATION

Synthesis of well-defined cyclic glycopolymers and the relationship between their physical properties and their interaction with lectins

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Cyclic polymers, which have a different topology from that of linear polymers, show potential as novel nanomaterials. In this commnuication, we report the synthesis of well-defined cyclic glycopolymers that have functional units for biomolecular recognition and evaluated their physical properties including molecular mobility. The suppressed molecular mobility of the cyclic glycopolymers was found to weaken their interactions with target proteins, demonstrating the influence of polymer topology on molecular recognition.

Topology is an important consideration in polymer science.[1] Cyclic polymers have structures with no chain-ends, and their molecular mobility is suppressed compared with that of their linear counterparts.[2–4] This imparts cyclic polymers with unique physical properties, such as higher glass transition temperatures, lower intrinsic viscosities, and smaller hydrodynamic volumes. Natural cyclic polymers (e.g., cyclic DNA and cyclic peptides) also exhibit specific biological functionalities, such as membrane penetration and high thermal stability, eliciting significant research interest in the field of nanoscience.[5,6]

Synthetic polymers are a class of nanomaterials that benefit from the availability of a versatile range of appropriate monomers.[7,8] However, while the functions of DNA and proteins (peptides) are based on their well-defined structures, the structures of synthetic polymers are heterogeneous in terms of their molecular weights, monomer sequences, and conformations. Endeavour for obtaining highly structurally precise synthetic polymers would provide opportunities for the development of novel nanomaterials with properties beyond those of biomacromolecules.[9]

Intermolecular interaction is a crucial function of biomolecules. Specifically, carbohydrate–protein interactions are vital in biological phenomena, as are protein–protein interactions.[10] For example, cell-surface glycoconjugates bind to their corresponding proteins (lectins) to induce physiological phenomena, such as immune response and pathogen infection.[11] Although the monomeric interaction between a terminal carbohydrate and a carbohydrate-recognition domain (CRD) of a lectin is weak (binding constant K_a = 10^{-3} M), multivalent binding between carbohydrates and the CRDs of a lectin enhances the total interaction (K_a > 10^5 M^{-1}).[12] This enhancement is known as the cluster glycoside effect.[13,14]

Synthetic polymers displaying glycoepitopes (glycopolymers) exhibit high affinity for target lectins and may be potential nanomedicines.[15–17] Recently, the synthesis of glycopolymers

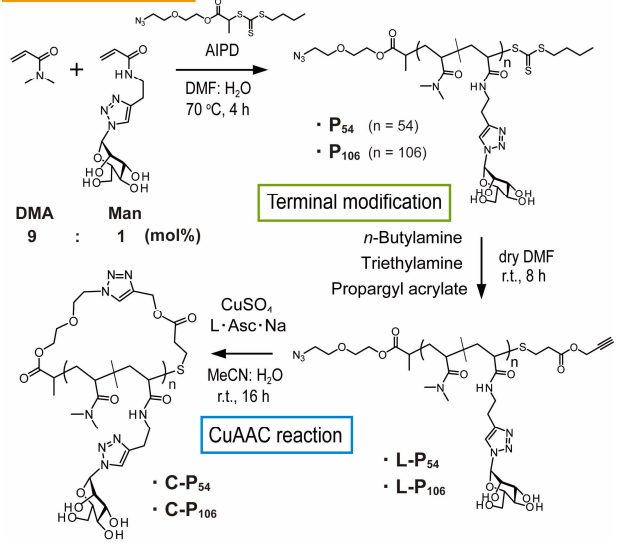

Figure 1. Synthetic scheme for the cyclic glycopolymers prepared in this study.

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†Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

Table 1. Properties of glycopolymers prepared by RAFT polymerization.[a]

Polymer	Target DP (mer)	Conv.[b] (%)	DP[b] (mer)	$M_{n,SEC}$[c] (g/mol)	$M_{w,SEC}$[c] (g/mol)	M_w/M_n[c]
P54	50	94	54	4,000	5,000	1.26
L-P54				4,100	5,200	1.27
C-P54				3,000	4,000	1.35
P106	100	98	106	8,000	10,900	1.37
L-P106				8,100	11,000	1.36
C-P106				7,200	9,800	1.35

[a]Monomer ratio of [DMA]: [Man] was set at 9: 1. Monomer concentration was 1 M in DMF: water (9: 1). [b]Monomer conversion and degree of polymerization (DP) were determined by ^{1}H NMR measurement. [c]Molecular weight and dispersity were determined by SEC analysis (*N*,*N*-dimethylformamide with 10 mM LiBr as eluent) calibrated to a polymethylmethacrylate standard.

with cyclic structures and their interactions with lectins have been reported.[18,19] However, the relationship between their physical properties, specifically molecular mobility, and their biomolecular interactions has not been widely discussed, despite the fact that the unique properties of cyclic polymers stem from their suppressed molecular mobility.

Herein, well-defined cyclic glycopolymers were synthesized to investigate the relationship between molecular mobility and function. The synthesis of well-defined cyclic glycopolymers was achieved by a combination of living radical polymerization and ring-closure strategies.[20] First, linear polymer precursors with the targeted molecular weight and narrow dispersities were prepared by living radical polymerization. Acrylamide-type monomers were used owing to their high propagating constants and water-solubilities.[21,22] Glycomonomer with mannose unit (Man) and *N*,*N*-dimethylacrylamide (DMA) were polymerized by reversible addition-fragmentation chain transfer (RAFT) polymerization. To enable the intramolecular cyclization of the polymer main chain by "click" reaction, the RAFT agent was modified with an azide moiety on the "R" group.[23–26] The [DMA]/[Man] monomer ratio was set at 9: 1 to avoid steric hindrance of the bulky glycounits during the cyclization reaction. The target degrees of polymerization (DP) were 50 and 100. The conversion rates of the polymerization as determined by proton nuclear magnetic resonance (^{1}H NMR) measurement were 94%

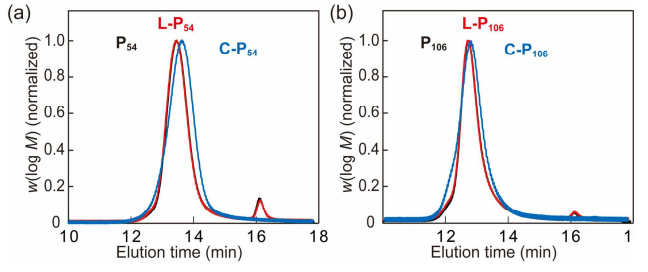

Figure 2. SEC chromatographs of the two series of glycopolymers. The eluent was DMF with 10 mM LiBr. The black, red, and blue lines indicate the glycopolymers with trithiocarbonate terminals, alkynyl terminals, and cyclic structures. The system was calibrated with polymethylmethacrylate standards.

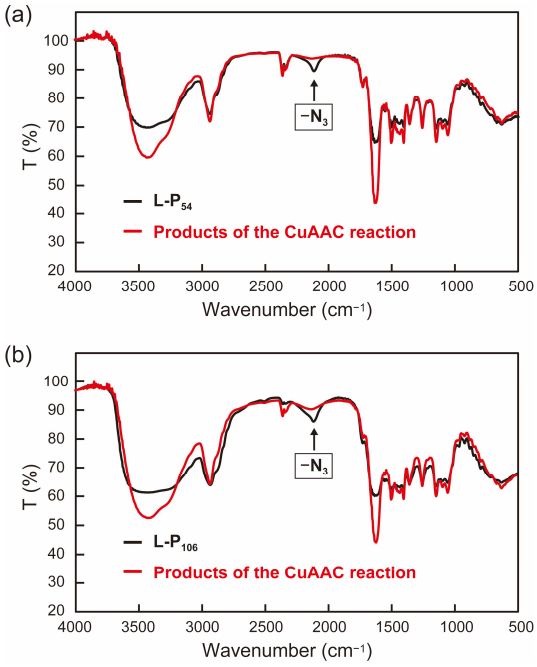

Figure 3. FT-IR spectra of the linear precursors and the crude cyclic polymers (before cycled SEC separation). The polymers with the degree of polymerization of 54 (a) and 106 (b). The black lines and red lines indicate the linear precursors and the crude cyclic polymers, respectively.

and 98% for DP = 50 and 100, respectively (Table 1). The DPs from ^{1}H NMR were 54 and 106 for the respective polymers (abbreviated to **P$_{54}$** and **P$_{106}$**). The relative molecular weights (M_n) and disperities (M_w/M_n) were determined by size exclusion chromatography (SEC) analysis. The M_n value for **P$_{106}$** (8,000 g/mol) was twice that of **P$_{54}$** (4,000 g/mol), showing the proportional relationship between molecular weight and DP. The M_w/M_n values for both polymers were lower than 1.25, indicating the precise control of the polymerization. After purification by reprecipitation, the trithiocarbonate terminals were converted to alkynyl groups. A one-pot procedure involving aminolysis and Michael addition successfully installed alkynyl groups onto the ω-terminals (**L-P$_{54}$** and **L-P$_{106}$**).[27] The progress of the reaction was confirmed both by the disappearance of UV absorbance at 310 nm and by appearance in the ^{1}H NMR spectra of a methylene-proton peak attributable to the position adjacent to the alkynyl group (Figure S13, S14 and S15). The terminal modification did not affect the molecular weights of the polymers (Table1 and Figure 2).

The ring-closure of the polymer main chain was realized by an intramolecular cyclization between an azide group on the α-terminal and an alkynyl group on the ω-terminal. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) in aqueous solution proceeded using a pseudo-dilution method to reduce the production of oligomeric by-products.[28] FT-IR measurement showed the disappearance of the peak of azide group at 2120 cm^{-1}, indicating that the CuAAC reaction almost completely proceeded[29] (Figure 3). The progress of the CuAAC reaction was also confirmed by the disappearance of the ^{1}H-NMR peak

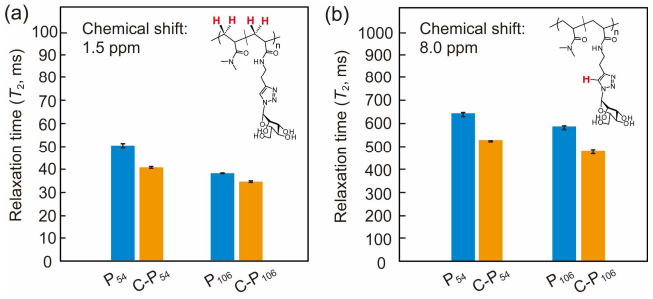

Figure 4. T_2 relaxation times for the glycopolymers at each chemical shift: (a) 1.5 and (b) 8.0 ppm in D_2O.

Table 2. Physical properties of the synthesized glycopolymers.

Polymer	D_h[a] (nm)	T_g (°C)	$T_2^{1.5\ ppm}$ [b] (ms)	$T_2^{8.0\ ppm}$ [b] (ms)
P_{54}	3.9±0.26	118	50.2	643
C-P_{54}	3.5±0.14	129	40.5	528
P_{106}	4.8±0.04	129	37.9	586
C-P_{106}	5.2±0.12	136	34.3	481

[a]Hydrodynamic diameter (D_h) was determined by dynamic light scattering (DLS) measurement (3 mg/mL in PBS solution). [b]Solvent was D_2O (10 mg/mL). [c]Not determined.

corresponding to the methylene protons adjacent to the alkynyl group (Figure S13 and S14). SEC chromatographs of the dialyzed sample revealed the existence of by-products with higher molecular weights than those of the linear precursors (Figure S16). The percentages of the by-products were calculated from peak-fitting analysis (Figure S17 and Table S2).[26] To purify the cyclic polymers, the oligomeric by-products were removed by cycled SEC separation (Figure S18 and S19). The higher-purity cyclic glycopolymers (**C-P_{54}** and **C-P_{106}**) showed the delay of peak-top elution compared with the linear precursors, which is a typical characteristic of cyclic polymers (Figure 2).[30] Furthermore, the molecular weights of the separated by-products were almost twice that of each linear precursor (Table S2 and Figure S20). These results support that the intramolecular cyclization through the CuAAC reaction provided the mixture of oligomeric by-products and the objective cyclic glycopolymers, and that the cyclic glycopolymers were successfully separated by the cycled SEC separation.

The physical properties of the synthesized cyclic glycopolymers were characterized by differential scanning calorimetry (DSC) and spin-spin (T_2) relaxation time measurement (Table 2). The DSC measurements revealed that the glass transition temperature (T_g) of the synthesized cyclic glycopolymers (**C-P_{54}** and **C-P_{106}**) are 129 and 136 °C, which are higher than those of their corresponding linear structures (**P_{54}** and **P_{106}** for 118 and 129 °C, respectively). The T_g values of the linear precursors bearing alkynyl groups were not determined due to the undesired coupling reaction between the polymer terminals during heating[31]. The lower T_g of the cyclic glycopolymers results from the elimination of the polymer terminals through intramolecular cyclization. This typical property of cyclic polymers promoted the formation of the cyclic structures of **C-P_{54}** and **C-P_{106}**. T_g reflects the molecular mobility of glycopolymers in a bulk state. To evaluate the molecular mobilities of the polymers in diluted solutions, the T_2 relaxation times of the glycopolymers at chemical shifts of 1.5 and 8.0 ppm, which correspond to the protons of the main chain and the proton of the triazole unit in the side chain, were measured (Figure 4). Longer T_2 relaxation times indicate higher segmental mobility of the polymer molecules.[32–34] The T_2 relaxation times of the cyclic glycopolymers (**C-P_{54}** and **C-P_{106}**) were shorter than those of the linear precursors (**P_{54}** and **P_{106}**). For both polymer length, the $T_2^{8.0\ ppm}$ values for the cyclic polymers were 0.82-times those of the linear precursors. The $T_2^{1.5\ ppm}$ of **C-P_{54}** was 0.81-times that of **P_{54}**, while the $T_2^{1.5\ ppm}$ of **C-P_{106}** was 0.91-times that of **P_{106}**. These results indicate that the molecular mobility of cyclic glycopolymers in a diluted state decreased due to the conformational restriction of polymer chains in cyclic structures. Furthermore, the degrees of mobility reduction for the side chains were similar for **P_{54}** and **P_{106}**, and the effect of molecular-mobility suppression of the polymer main chain was greater for short polymer lengths.

Molecular recognition of the synthesized glycopolymers was evaluated by hemagglutination inhibition (HI) assay.[35,36] Concanavalin A (ConA) is a tetrameric lectin with an affinity for mannose units and causes the aggregation of red blood cells (RBCs) through binding to the mannose units on the surface of RBCs. The synthesized glycopolymers presenting mannose units can bind to ConA and inhibit this aggregation. The minimum polymer concentration required for hemagglutination inhibition is determined as K_i. Lower K_i values indicate stronger interaction with ConA. The K_i value of **C-P_{54}** was 730 μM while those of **P_{54}** and **P_{106}** were 55 and 99 μM, respectively (Table3 and Figure S23). The K_i value of **C-P_{106}** was not detected in the experimental concentration range. The cyclic glycopolymers showed higher K_i values than those of the corresponding linear polymers, demonstrating the weaker interactions with ConA.

The interactions of the glycopolymers with ConA were also evaluated by fluorescence quenching measurement with fluorescein isothiocyanate labelled ConA (FITC-ConA).[35,37,38] FITC groups have an intrinsic emission peak at 517 nm that is quenched upon binding to glycoepitopes. The fluorescence intensity change ΔF (F_0–F_n) at each polymer concentration was measured. The addition of the glycopolymers (**P_{54}**, **P_{106}** and **C-P_{106}**) into the FITC-ConA solution caused the quenching of FITC fluorescence, while the addition of **C-P_{54}** did not (Figure 5). The plots were analyzed using the Langmuir adsorption isothermal

Table 3. The K_i values of the glycopolymers against ConA.[a]

Polymer	K_i (μM)
P_{54}	55
C-P_{54}	730
P_{106}	99
C-P_{106}	n.d.[b]

[a]The minimum mannose concentration required for HI activity (μM). [b]Not determined (no activity in the concentration range).

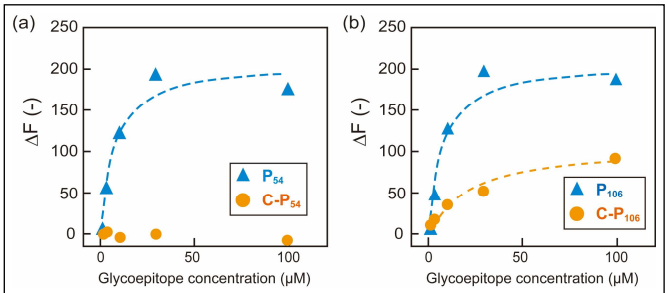

Figure 5. Quenching of fluorescence intensities (FITC-ConA) by the addition of glycopolymers. **P_{54}** and **C-P_{54}** for (a), and **P_{106}** and **C-P_{106}** for (b).

model. The binding constants (K_a) of **P_{54}**, **P_{106}** and **C-P_{106}** were 1.43×10^5, 1.21×10^5, and 4.29×10^4 M^{-1}, respectively. These results indicate that the interactions of the cyclic glycopolymers were weaker than those of the linear glycopolymers.

The results from the above two experiments demonstrated that the cyclic glycopolymers showed weaker interactions with ConA compared with the corresponding linear glycopolymers. Although the compositions of the polymer structures were the same, the difference of the polymer topology affected the molecular recognition of the glycopolymers. It is noteworthy that larger glycopolymers tend to exhibit better interactions with lectins owing to their ability of multivalent binding to two of the four CRDs in ConA.[35,36,39] However, since the hydrodynamic diameters (D_h) of **P_{106}** and **C-P_{106}** are larger than those of **P_{54}** and **C-P_{54}** (Table 2, Figure S21 and S22), one can exclude the possibility that the smaller molecular size of the cyclic glycopolymers causes the difference in the molecular interactions. As shown in Figure 4, T_2 relaxation time of the glycopolymers were decreased by cyclization of the polymer main chains. The lower T_2 values indicate slower segmental motion of the polymers, and the relationship between shorter T_2 relaxation time and weak molecular interaction was observed for linear glycopolymers in our previous report.[36] Although the order of T_2 values is irrelevant to the order of the strength of the molecular interactions, the weak interactions of the cyclic glycopolymers with ConA can be caused by the suppressed molecular mobility of the glycoepitopes in the side chains.

Conclusions

In summary, cyclic glycopolymers with defined structures were synthesized, and the relationship between their molecular mobilities and interactions with ConA were discussed. The synthesized cyclic glycopolymers exhibit typical properties of cyclic polymers, with delayed peak-top elution in SEC analysis and higher grass transition temperatures compared with those of their linear counterparts. The segmental mobilities of the glycopolymers in diluted solutions were evaluated by T_2 relaxation time measurement. The cyclic glycopolymers showed shorter T_2 relaxation times, reflecting their suppressed molecular mobility. In the HI assay and the fluorescence quenching measurement, the interactions of the cyclic glycopolymers with ConA were shown to be weaker than those of their linear precursors. This suggests that the cyclization of the polymer main chains weakens the interaction abilities of the glycopolymers by suppressing molecular mobility. The correlation between the molecular mobility of the cyclic polymers and their molecular recognition should be further investigated. This work revealed that the molecular recognition of biomolecules by synthetic polymers can be controlled by topological design of the polymer structures. Accordingly, this study will contribute to the development of novel biomedical materials with protein-repelling or high-membrane-penetration properties.

This work is supported by JSPS (22K14728, 22H05048, and 19H02766).

Author Contributions

The experiments were planned and conducted by M. Nagao. The manuscript was written by M. Nagao. Y. Miura and Y. Hoshino made a discussion and provided the advice to improve the quality of the research.

Conflicts of interest

There are no conflicts to declare.

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