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Communication

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Switchable Binding Affinity of Mannose Tethered to Collagen Peptide by Temperature-Dependent Triple-Helix Formation

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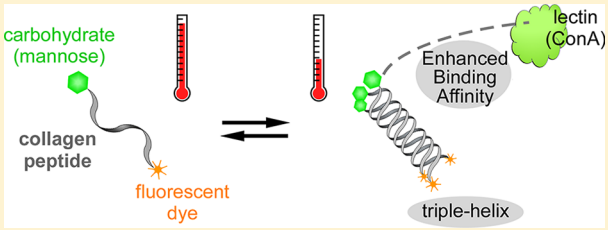
Supporting Information

ABSTRACT: Novel glycopeptides were created with a view to regulate the bindings of carbohydrates to lectins as a means of controlling biological function. We synthesized glycopeptides containing mannose (Man) tethered to a collagen peptide moiety (MPOG10: $-(\text{Pro-Hyp-Gly})_{10}-$ or MGPP10: $-(\text{Gly-Pro-Pro})_{10}-$). Circular dichroism spectra showed formation of a triple helical structure for MPOG10, and the melting temperature indicates that MPOG10 forms a more stable triple helical structure than MGPP10 in phosphate buffered saline (PBS). At 25 °C, fluorescence polarization (FP) values of MPOG10 and MGPP10 increased following the addition of *concanavalin A* (ConA), and the addition of α-methyl-mannose (MeMan) to a mixed solution of each glycopeptide with ConA resulted in a decrease in FP values. These results confirm that the previous increase in FP values observed was caused by ConA binding to Man on MPOG10 or MGPP10. The binding affinity of MPOG10 was higher than that of MGPP10, and the dissociation constant of MPOG10 to ConA was 1.9×10^{-5} (mol/L). The observed binding of MPOG10 to ConA at 25 °C was reduced at higher temperature (50 °C). Therefore, the enhanced binding affinity of MPOG10 to ConA could be accounted for by formation of a clustered Man moiety triggered by the formation of a more stable triple helical structure of MPOG10 compared with MGPP10.



Binding events of biomolecules contribute to a variety of biological functions such as those involving immune systems, cell adhesion, and infection processes. The ability to control the affinity properties of biomolecules can potentially be utilized as a tool in various medical therapeutic strategies. For example, researchers have recently developed functional molecules that have the ability to control or switch binding events between functional biomolecules. These molecules utilize conformational changes, temperature-dependent polymer aggregation, or stimulant-responsive structures.[1−5] Lim et al. reported glycoconjugate nanoribbons to control bacterial cell cluster formation, which is induced by their self-assembly.[6]

Carbohydrates represent one group of biomolecules that possess biological activity.[7−9] Despite great interest, researchers continue to face difficulties in elucidating the binding properties of various carbohydrates given the generally weak binding affinities. Efforts to enhance the binding affinities of carbohydrates have made use of what is referred to as the "clustering effect".[7,10−13] The clustering effect is an observed phenomenon whereby the association constant of a clustered carbohydrate is greater than that of the nonclustered carbohydrate. A number of molecular probes utilizing the clustering effect have been applied as the sensing probes to analyze the binding properties of carbohydrates.

In this research, we developed a novel glycopeptide as a molecular tool to control the binding affinity of carbohydrates as utilizing the clustering effect triggered by triple-helix formation of a collagen peptide chain. Since binding events between carbohydrate and protein cause biofunctional reaction such as endocytotic uptake or virus infection, the glycopeptide that enables control of their binding affinities is a promising molecular tool to accelerate biomedical research and to apply to a diagnostic clinical medicine.

Novel glycopeptides were designed and synthesized with the aim of regulating the binding of carbohydrate to lectin. We designed two types of glycopeptides (MPOG10 and MGPP10) as shown in Figure 1. Both possess a carbohydrate (Man: mannose) moiety and fluorescent dye (NBD: nitrobenzofurazane) tethered to a collagen peptide. Since collagen peptides H-$(\text{Pro-Hyp-Gly})_{10}$-OH and H-$(\text{Gly-Pro-Pro})_{10}$-OH reportedly form triple helical structures, glycopeptides containing the collagen sequences can potentially self-assemble to form triple helical structures.[14−16] In our case, the triple-helical structure would give a filament with three Man moieties at the terminal end of each strand, thus inducing a clustering effect. As triple-helix formation equilibrates, a switchable regulation system for the binding of carbohydrate to lectin could be achieved.

The glycopeptides were obtained using liquid- and solid-phase syntheses. With the liquid-phase synthesis, building blocks (Fmoc-Pro-Hyp-Gly-OH, Fmoc-Gly-Pro-Pro-OH, Z-Ser(Ac_4Man)-OH, and Fmoc-Lys(NBD)-OH) were initially synthesized, and these were then applied to the Fmoc-based

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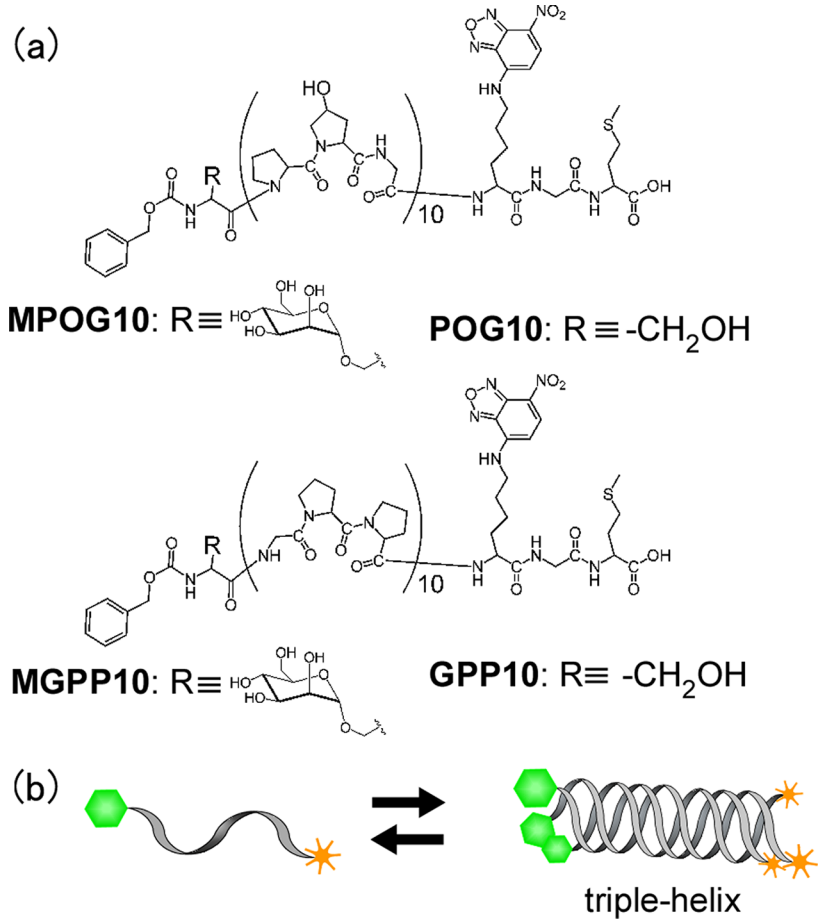

Figure 1. (a) Structures of glycopeptides (MPOG10, POG10, MGPP10, and GPP10) and (b) schematic representation of triple-helix formation.

solid-phase synthesis to obtain MPOG10 and MGPP10. Glycopeptides without Man, POG10, and GPP10 were also prepared as reference molecules. For MPOG10 and POG10, the coupling reactions were carried out with heating in order to overcome steric hindrance. The synthesized glycopeptides were purified by HPLC and identified by mass spectroscopy (see SI for details of the synthetic procedures).

The stability of the triple helical structure for MPOG10, POG10, MGPP10, and GPP10 was examined by circular dichroism (CD). The CD spectra of MPOG10 and POG10 (at 5 °C) showed a positive peak and a negative peak at 225 and 200 nm, respectively (Figure 2a). These peaks were absent in spectra of MGPP10 and GPP10 or showed a negligible positive peak at 225 nm. The presence of both positive and negative peaks in the spectra of MPOG10 and POG10 indicates formation of a triple helix, unlike the case with MGPP10 and GPP10. According to a previous study, the intensity ratio of the positive and negative peaks (Rpn value) is a measure of triple-helix formation.[15−17] The Rpn value for both MPOG10 and POG10 was 0.11, while the value for natural collagen is 0.12.[18] This confirmed that MPOG10 and POG10 formed triple helical structures. Moreover, the melting temperature (T_m) of MPOG10 and POG10 was 58 and 62 °C, respectively, while the reported T_m of H-(Pro-Hyp-Gly)$_{10}$-OH is 59 °C.[19] Since the T_m values of MPOG10 and POG10 are similar to that of the free glycopeptide, terminal modification of the glycopeptides with Man and NBD does not disrupt triple-helix formation. Additional evidence of the triple-helix structure of MPOG10 and POG10 was obtained by transmission electron microscopy (TEM). The TEM image of MPOG10 (Figure 3) shows unique filament objects with width in the range of 1.0−3.0 nm. The histogram showing the width distribution shows that the filament width is ca. 1.6 nm. This is consistent with MPOG10 adopting a triple helical structure, as the reported width of a triple helical collagen peptide was 1.5 nm as determined from X-ray structural analysis.[20,21] Although similar filaments are observed in the TEM images of POG10 (see SI), no characteristic filaments are observed in the TEM images of MGPP10 and GPP10. These results are consistent with those obtained from the CD analysis, and confirm that MPOG10 and POG10 can each form a stable triple-helix structure, unlike the case with MGPP10 and GPP10.

The binding affinity of the glycopeptides to lectin was examined by fluorescence polarization (FP).[22,23] Prior to the affinity evaluation an annealing step was employed, which comprised heating of the glycopeptide solution to unfold the peptide, followed by slowly cooling to facilitate formation of the thermodynamically stable filament. When ConA (5 μmol/L) was added to MPOG10 (30 μmol/L), the FP value increased by 25.9 ± 12.7 (mP), while no change was observed in the fluorescence emission spectra. In the case of POG10, addition of ConA caused an increase in the FP value by 12.9 ± 8.3 (mP), although the increase in FP values observed with MPOG10 and ConA is larger, as shown in Figure 4. Since the increase in FP value for MPOG10 and MGPP10 is larger than that for POG10 and GPP10, ConA binds to MPOG10 and MGPP10 with higher affinity than to POG10 and GPP10. In an effort to estimate the amount of glycopeptides displaced by

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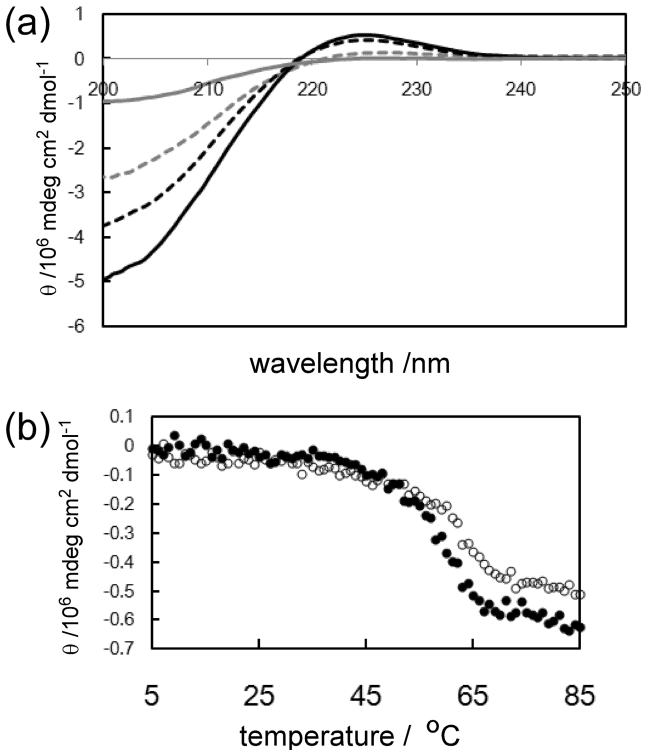

Figure 2. (a) CD spectra (at 5 °C) and (b) molar ellipticities at 225 nm (5−85 °C) for each glycopeptide: MPOG10, black solid line and filled circle; POG10, black dotted line and open circle; MGPP10, gray solid line; GPP10, gray dotted line. The concentration of each glycopeptide is 2.0×10^{-4} mol/L in PBS.

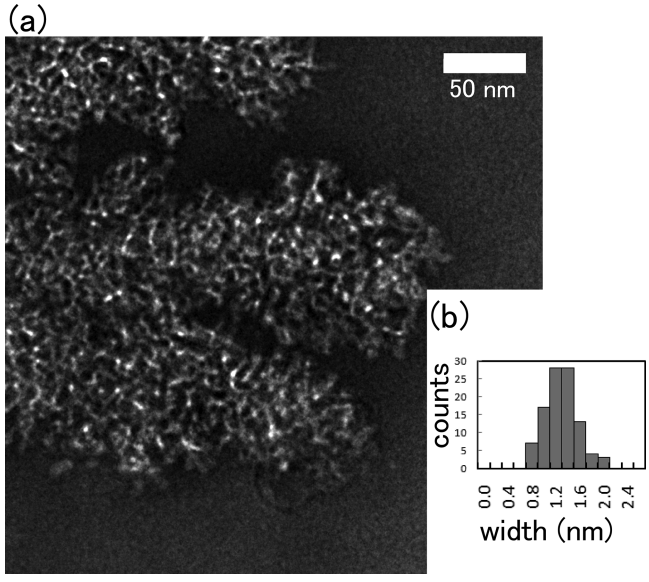

Figure 3. (a) TEM image of MPOG10 on a carbon-coated copper microgrid (200 kV, negatively stained with phosphotungstic acid) and (b) histogram of the width distribution of the filament (n = 100).

measuring the decrease in FP value, MeMan was added to a solution of ConA and MPOG10 or MGPP10 and FP values were measured.[24] When 4.5 mmol/L of MeMan was added, the FP values of both MPOG10 and MGPP10 decreased. The displacement ratio was calculated as 29% for MPOG10 and 48% for MGPP10; hence, the displacement of MGPP10 with MeMan was greater than that of MPOG10. These results

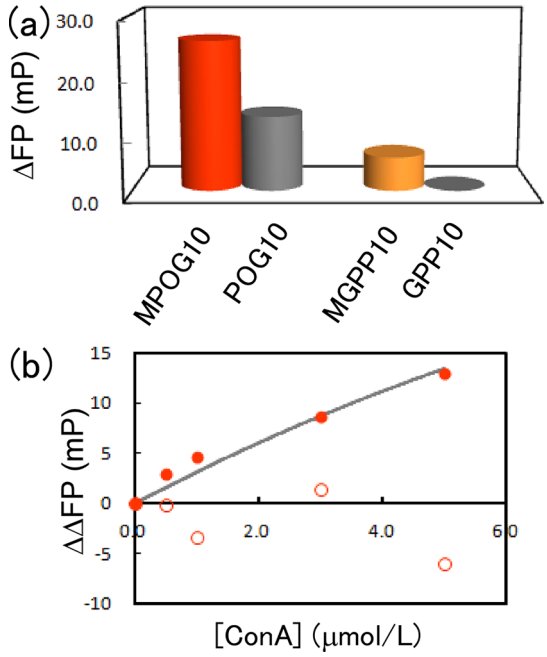

Figure 4. (a) Changes in fluorescence polarization (ΔFP) of glycopeptides (MPOG10, POG10, MGPP10, and GPP10) following addition of ConA (5.0×10^{-6} mol/L) and (b) differential of the changes ($\Delta\Delta FP = \Delta FP_{MPOG10} - \Delta FP_{POG10}$) following addition of ConA ((0−5.0) × 10^{-6} mol/L) at 25 °C (filled circles) and 50 °C (open circle). The concentration of each glycopeptide is 3.0×10^{-5} mol/L in PBS.

ensure that the increased FP values observed for MPOG10 and MGPP10 are derived from ConA binding to Man on MPOG10 and MGPP10, and that the binding of ConA to Man on MPOG10 is greater than the binding of ConA to Man on MGPP10. The higher affinity of MPOG10 than MGPP10 is consistent with the stability of the triple helical structure that MPOG10 is more stable than MGPP10.

The dissociation constant (K_d) associated with the binding of ConA to Man on MPOG10 or MGPP10 was examined with changing concentration of ConA from 0 to 5.0 μmol/L. With the solution containing MPOG10, the FP values increased with increasing concentration of ConA (Figure 4b), and the K_d value calculated by nonlinear least-squares fitting[25−27] was 1.9×10^{-5} (mol/L). With the solution containing MGPP10, the FP values increased with increasing concentration of ConA; however, the K_d value could not be calculated using nonlinear least-squares fitting since the increment in FP was too small and did not achieve convergence. The lack of convergence within the examined concentration range of ConA confirms that the K_d for Man on MGPP10 is more than 10^{-4} (mol/L). Since the K_d of MGPP10 is similar to the reported K_d (ca. 10^{-4} mol/L)[28] for free mannose (not clustered) to ConA, mannose tethered to GPP10 would hardly obtain the clustering effect. These results prove that the binding affinity of MPOG10 to ConA is higher than that of MGPP10. The enhanced binding affinity of MPOG10 is speculated to result from a clustering effect derived from the Man moieties and triggered by formation of the triple-helix.

Moreover, effect of the triple-helix formation on the enhanced affinity was examined by the FP measurement under 50 °C. Denature of ConA was not observed at 50 °C which was preliminarily confirmed by CD analysis. At higher

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temperature (50 °C), the ΔFP value of MPOG10 (ΔFP_{MPOG10}) is similar or lower than that of POG10 (ΔFP_{POG10}) to give small or negative ΔΔFP value as shown in Figure 4b. When 3 μmol/L of ConA was added, the ΔΔFP at 25 °C is 6-fold larger than ΔΔFP at 50 °C. These positive ΔΔFP values observed exclusively at 25 °C indicate that the triple-helix formation triggers the clustering effect of Man moieties. The negative ΔΔFP value at 50 °C suggests that ConA at higher density promotes an unthreading event of MPOG10. Such sequence- and temperature-dependent affinities of Man proved that the affinity of Man could be enhanced by a collagen peptide that forms stable triple helical structure.

In summary, novel glycopeptides comprising carbohydrate tethered to a collagen peptide motif (MPOG10, MGPP10, POG10, and GPP10) were synthesized. The CD and TEM analyses confirmed that MPOG10 and POG10 adopt a triple helical structure more favorably than MGPP10 and GPP10. Affinity evaluation using FP analysis showed that Man on MPOG10 or MGPP10 binds to ConA, and that the binding affinity of MPOG10 is higher than that of MGPP10. The enhanced binding affinity is speculated to arise from the presence of a clustered Man structure that is triggered by collagen peptide forming a stable triple helix. The enhanced affinity of MPOG10 was decreased by raising the temperature which is an unfavorable condition for MPOG10 to form a stable triple-helix. These results have proven that the affinity of Man tethered to collagen peptide is controllable by temperature and peptide sequence. This study would contribute to the potential use of glycopeptides as molecular tools for regulating the binding affinity of carbohydrates.

■ ASSOCIATED CONTENT

Supporting Information

Details of synthetic procedures, characterization (NMR and MS), and affinity evaluation methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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