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Folding Control of Non-natural Glycopeptide Using Saccharide-coded Structural Information for Polypeptide

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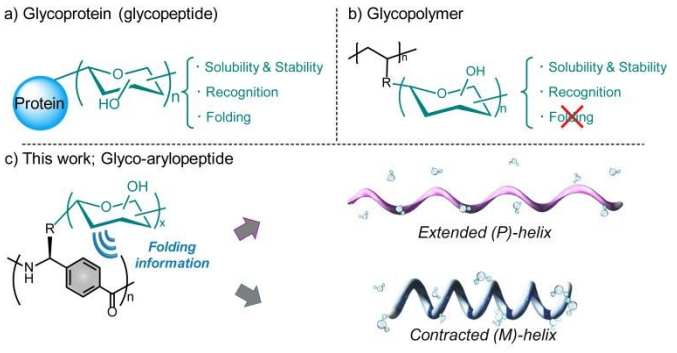
We synthesized "glyco-arylopeptide", whose folding structure significantly changes depending on the kind of saccharide in side chain. the saccharide moiety interacts with the main chain via hydrogen bonding, and the non-natural polypeptide form two well-defined architectures—(*P*)-3_1- and (*M*)-4_1-helices—depending on the length of the saccharide chains and even the configuration of a single stereo-genic center in the epimers.

Saccharides are the foundation of living systems, and glycosylated polypeptide (glycopeptide) plays a unique role in a series of biological events. The saccharide moieties not only determine the physicochemical properties, such as solubility and stability of polypeptides, but also provide significant information about the folding/unfolding of polypeptides and molecular recognition involving other molecules (Scheme 1a).[1] These functions have inspired chemists to utilize saccharide units in artificial polymers, including the peptide backbone.[2] In general, synthetic glycopolymers are known to show enhanced interactions with cells and have potential applications in cell sensing,[3] drug delivery,[4] molecular recognition,[5] etc. (Scheme 1-b).[6] However, there are only a few studies that have investigated the role of saccharide structures in the folding behavior and stability of glycopolymers and extracted information related to folding,[7] although lectins rigorously distinguish the slight differences between monosaccharides and disaccharides in saccharide chains *in vivo*. The reason is that the saccharides have similar hydrophilic properties and may only differ in the number of OH groups and configuration of a single or a few stereogenic centers from the standpoint of organic chemistry. Therefore, it is difficult to design an artificial molecular system that recognizes slight structural differences in saccharides.

Recently, we reported an aromatic polyamide peptide, poly-"arylopeptide", which comprises aromatic amide spacers between each residue.[8] Arylopeptides show structural changes depending on their chemical structures. Therefore, we surmised that the arylopeptide might be able to extract the folding information from saccharide substituents similar to natural glycopeptide. Herein, we present a glycosylated arylopeptide called "glyco-arylopeptide". This arylopeptide could recognize the saccharide moieties depending on the length of the saccharide chain and the epimers with the slight differences in the configuration of a single stereogenic center to form discrete well-defined structures (Scheme 1c). In addition, the folding information varied reversibly/irreversibly because of the chemical modification same as in nature and the recognition of other molecules.



Scheme 1. Schematic diagram of (a) glycoprotein and (b) glycopolymer. (c) This work; Chemical structure of glycol-arylopeptide and folding control using saccharide-coded folding information.

As shown in Scheme 2, we synthesized glycoarylopeptides bearing various saccharide derivatives (**poly-1a–d**). All reactions were done by using the optimized procedures,[9] and they proceeded quantitatively. Experimental details and full characterization of the products are presented in the

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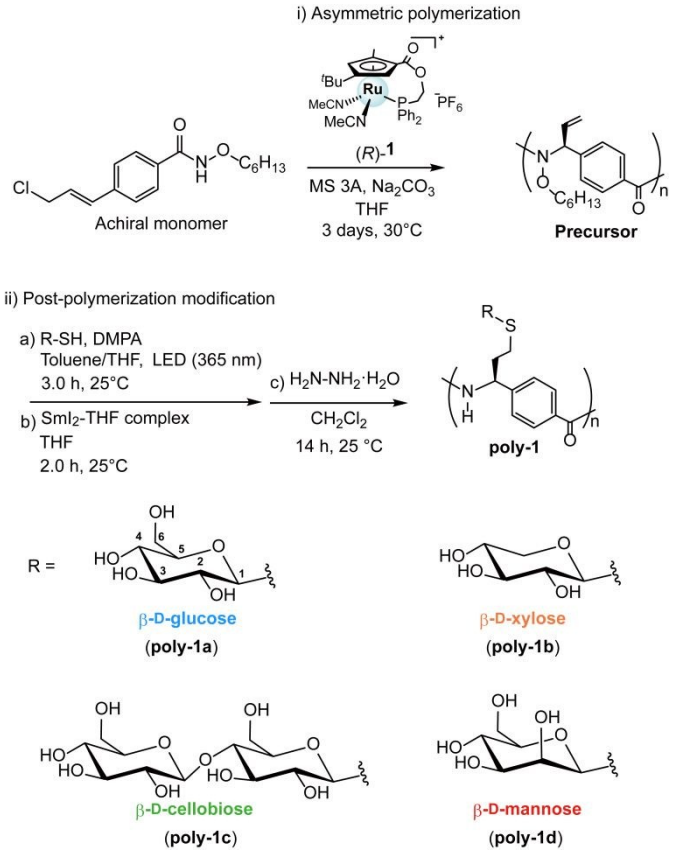
Supporting Information (SI). **Poly-1a–d** are soluble in ordinary solvents, such as THF, acetonitrile, ethanol, and water.[10]



Scheme 2. Synthetic pathway of glycoarylopeptides

To investigate the global conformation of **poly-1**, circular dichroism (CD) spectroscopy was conducted using water as the solvent. As shown in Fig. 1a, CD spectra of **poly-1a** in organic solvents showed a plus to minus bisignate Cotton effects, indicating the formation of (*P*)-helix caused by the asymmetric carbon (*S*) on the main chain. This is in accordance with our previous work.[9] In water, however, the CD spectrum of **poly-1a** presented the opposite bisignate Cotton effect between 240 and 259 nm (Fig. 1a, blue line). The computational analysis of the oligomers suggested that the minus to plus Cotton effect corresponded to the (*M*)-twisted conformation (Fig. 1b). The CD spectra obtained from CD simulation of oligomers (n = 2) in water and THF by using TD-DFT were very similar to the observed spectra for **poly-1a**. The major difference between the optimized structure of (*P*)-twist and (*M*)-twist is the reversal in the sign of dihedral angles (ϕ, θ) in the local structures and the formation of cyclic intramolecular C6-OH···O=C hydrogen bonds in the (*M*)-twist (Fig. 1b and Fig. S47d).

To study the role of saccharide structure, CD spectra of the analog were measured in water. The CD spectrum of **poly-1b** without C6-OH showed ordinary (*P*)-conformation (Fig. 2a). It is surprising that **poly-1c** having D-cellobiose, a disaccharide of glucose, also formed the (*P*)-conformation, although the D-glucose moiety is connected to the main chain in the same manner as that in **poly-1a**. The C6-OH of D-cellobiose in the optimized structure was easily incorporated into the hydrogen bond with the adjacent pyranose ring and could not interact with the main chain (Fig. S53). Interestingly, **poly-1d** also formed the (*P*)-conformation with the epimer of D-glucose at the C2-position. The axial C2-OH of mannose and its hydration probably disrupts the preferred conformation of carbohydrate for (*M*)-helix (Fig. S54).[11] In glycopeptide, a few saccharides near the glycosylated site are known to determine the stability of the peptide backbone via hydrogen bonding between the saccharide and the carbonyl group on the main chain.[12] Therefore, the intramolecular hydrogen bonding of D-glucose in the glycoarylopeptide would also play an important role in the folding behavior similar to that observed in glycopeptides. In other words, the backbone of arylopeptide identifies the length of the saccharide-chains and the epimer and hence can receive distinct folding information from each saccharide.

The above experimental results and computational simulations lead us to propose two global conformations (Figs. 2b and 2c)—(*P*)-helix and (*M*)-helix. The (*P*)-helix is 3_1-like helical structure formed by successive formation of a (*P*)-twist with a pitch of 5.1 Å. The (*M*)-helix is more contracted 4_1-like helix with a pitch of 3.0 Å. The structural study using atomic force microscopy (AFM) revealed detailed insights the glycopeptide.[13] The AFM images of **poly-1a** film, cast using pyridine/THF solution on mica, exhibited a height of 0.9 ± 0.1 nm (Fig. 2d). This matched well with the height of the proposed structure of **poly-1a**, supporting the estimated structures of Fig.

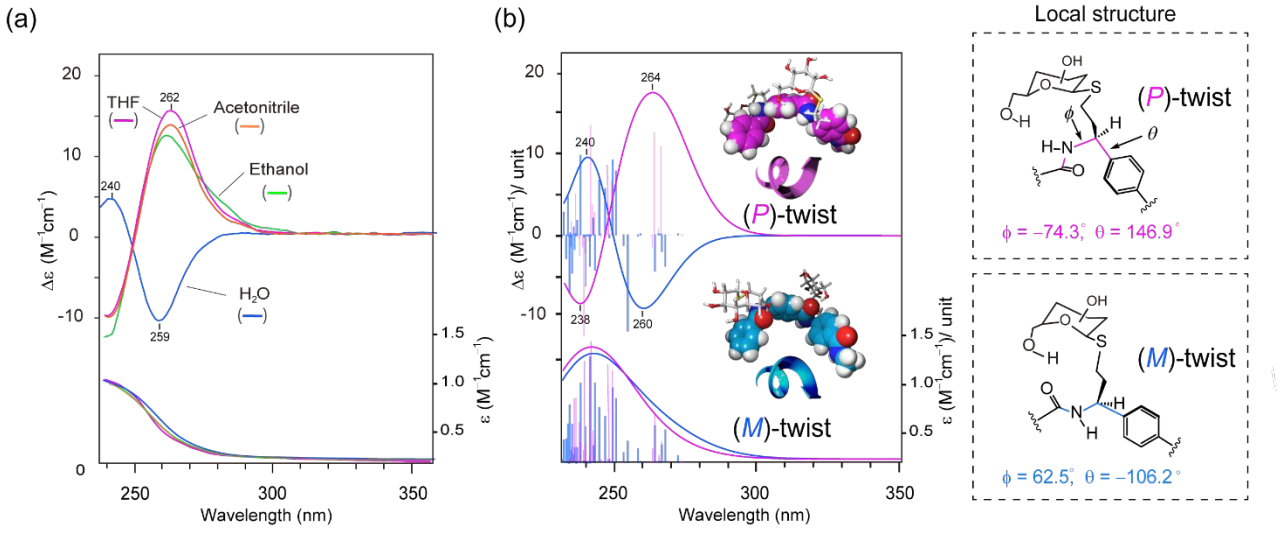


Fig. 1 (a) Solvent effect of CD spectra of **poly-1a** in 1% (v/v) DMSO/(solvent) at 298 K. Solvent; THF (pink), acetonitrile (orange), ethanol (green) and H_2O (blue). Concentration: [**poly-1a**] = 0.30-0.32 mM. (b) Simulated Structures and CD spectra of oligomer (n = 2), (*P*)-twist and (*M*)-twist by TD-DFT calculation (B3LYP/6-31**) in THF (pink curve) and water (blue curve) and their local structures.

2b and Fig. 2c. The AFM images of **poly-1a** cast using pyridine/H_2O solution showed nanoparticles, therefore no useful information was from these data.

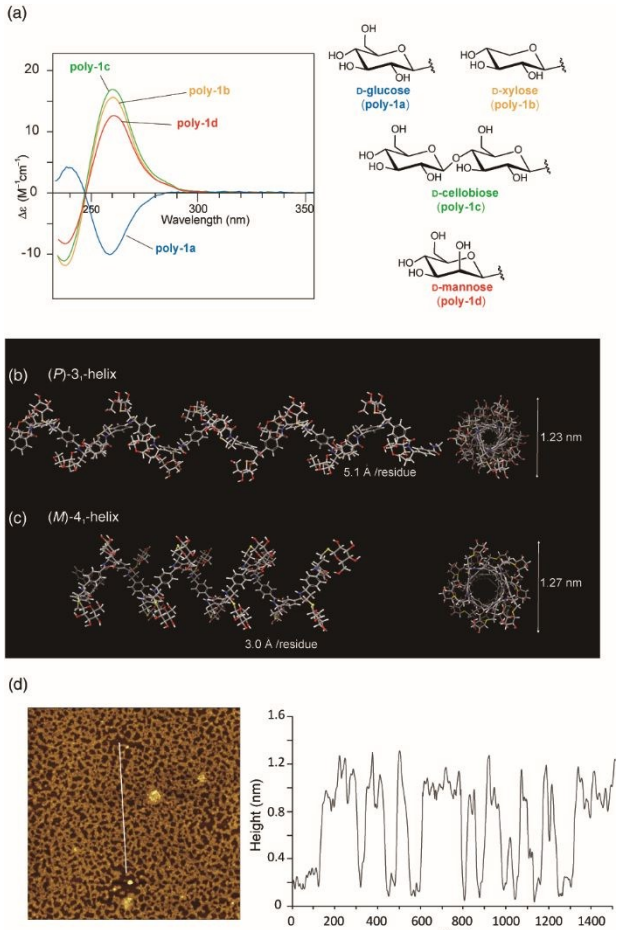


Fig. 2 (a) CD spectra of **poly-1a**-**d** in 1% (v/v) DMSO/H_2O at 25 ºC. Concentration: [**poly-1a**] = 0.31 mM, [**poly-1b**] = 0.30 mM, [**poly-1c**] = 0.37 mM, and [**poly-1d**] = 0.38 mM. Two plausible 3D helical conformations of **poly-1a** (n = 17) in (b) THF and (c) water. (d) AFM image of **poly-1a** (3.0 um ×3.0 um, topography) and the height profiles.

L-Amino acid residues induce only a right-handed α-helix; however, the residues of glycoarylopeptide form helices with both screw-senses.[14] Thus, the helical stability of **poly-1a** was investigated in detail. The molecular weight-dependences of the helical formation were examined by CD measurement in THF and H_2O. The CD intensity of **poly-1a** increased nonlinearly as a function of the degree of polymerization (*n*) and plateaued at higher *n* values (Fig. S44a). The results suggest that a long polymer chain fixes the (*P*)- and (*M*)-conformation.[15] The temperature dependence of the helices indicated that the (*M*)-helix diastereomer is a more unstable than the (*P*)-helix diastereomer (Fig. S44b).[16] Actually, when THF was added to the solution of **poly-1a** in H_2O, (*M*)-helix changed suddenly to (*P*)-helix (Fig 3a). On the other hand, the addition of H_2O to (*P*)-helix in THF did not adopt the (*M*)-helix (Fig. S40). The dynamic behavior of glycoarylopeptide including helical inversion is a unique character that is not seen in previously reported arylopeptides.[8a]

Natural glycopeptides change their folding structures and functions through various modifications or interactions with other molecules and saccharide moieties. The OH groups of saccharides are frequently acetylated to change the chemical and biological properties *in vivo*.[17] Thus, acetylation and deacetylation of the OH groups on **poly-1a** were carried out. The CD spectrum of the acetylated **poly-1a** showed a plus to minus Cotton effect originating from the (*P*)-helix (Fig. 3b). The other acetylated glycoarylopeptides (**poly-1b-d**) showed no inversion in Cotton effect, implying that the acetyl groups disrupted the delicate energy balance of the (*M*)-helix in **poly-1a**, and thus, the folding information for (*M*)-helix was manipulated. Subsequently, the structural transitions caused by molecular recognition were also investigated. Boronic acid reagent is known to show favorable interactions with saccharides.[18] Adding the 4-carboxy-phenylboronic acid to the **poly-1a** in aqueous solution resulted in a drastic change (consistent with the helix-to-helix transition) from the contracted (*M*)-4_1-helix to the extended (*P*)-3_1-helix (Fig. 3c). The CD spectrum changes presented in Figs. 3a and 3c show an isodichroic point, indicating the existence of mainly two kinds of chiral architectures; (*P*)-helix and (*M*)-helix.

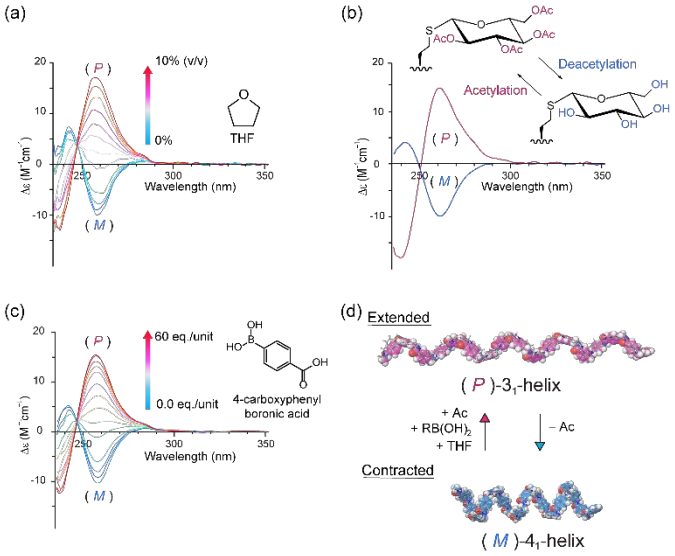


Fig. 3 CD spectra of (a) **poly-1a** in the mixture of H_2O and THF, and (b) **poly-1a** with acetylation and deacetylation. ([**poly-1a**] = 0.31 mM, 1%DMSO/H_2O solution, 298 K). (c) CD titration experiment of **poly-1a** in H_2O at 298 K in the presence of 0, 4, 8,..., 60 equiv of 4-carboxyphenylboronic acid. (Initial concentration: [**poly-1a**] = 0.25 mM, 1%DMSO/ 10 mM NH_3 solution, pH = 10.5). (d) The image picture of helix to helix transition of **poly-1a**. The chain-length from (*M*)-4_1-helix to (*P*)-3_1-helix extend about 1.7 times of its length (pitch per residue; [(*P*)-3_1-helix] 5.1 Å and [(*M*)-4_1-helix] 3.0 Å). Side chains are omitted for clarity.

In conclusion, we succeeded in establishing a folding control in a glycoarylopeptide using saccharide-coded folding information for a peptide backbone. The arylopeptide distinguished the configuration of a single stereogenic center in the epimers—D-glucose and D-mannose—and formed a completely distinct and well-defined architecture ((*P*)-3_1-helix or (*M*)-4_1-helix) in an aqueous solution. Similar to the recently reported mechanism for glycopeptides,[11] the saccharides near the glycosylated site would determine the properties of the peptide backbone. (*P*/*M*)-helix was easily converted to the other structure via simple chemical modification (acetylation/de-acetylation) or by using additives. These results indicate that the properties of completely artificial polypeptide

molecules are also dominated by the type of saccharides; this is analogous to the behavior of natural polypeptides. Further studies are in progress for establishing an elaborated relationship between peptide backbone and the saccharides in terms of chiral information and hydration.

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