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Glyco Star Polymers as Helical Multivalent Host and Biofunctional Nano-Platform

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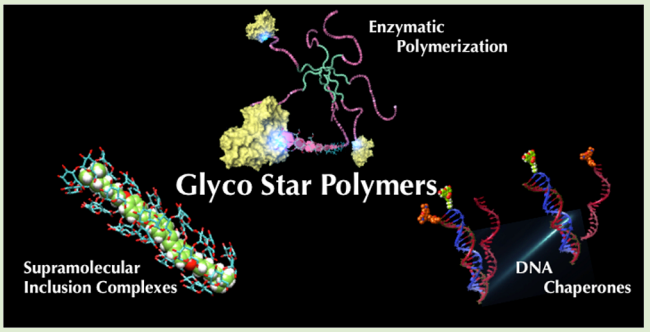
Supporting Information

ABSTRACT: A series of amylose-based star polymers (1, 2, 4, and 8 arms) as a new glyco biomaterial was synthesized by a click reaction and enzymatic polymerization of specific primers with phosphorylase. The molecular weights were controlled by the enzymatic reaction. Further polymerization resulted in a viscous solution and, especially, for the 8-arm primer, a hydrogel was obtained due to effective cross-linking between the multiarmed structures. The star polymers with a degree of polymerization of about 60 per arm acted as an allosteric multivalent host for hydrophobic molecules by helical formation. A cationic 8-arm star polymer catalyzed DNA strand exchange as a nucleic acid chaperone. Amylose-based star polymers are promising building blocks for producing advanced hybrid glyco biomaterials.



Natural polysaccharides are structurally and functionally diverse molecules. Amylose and cellulose are well-known homolinear glucose polymers, while other polysaccharides exist as linear block copolymers (e.g., alginic acid), graft copolymers (e.g., xanthan), and spherical branched polymers (e.g., glycogen). This structural diversity, which is elaborately designed by nature, confers the polymers with specific physical properties applicable for specific functions.[1] Consequently, adding a unique structural feature to polysaccharides is expected to provide hybrid polymers with advanced functionality and expanded applications.

Numerous polysaccharides (e.g., cellulose, chitosan, dextran, and hyaluronan) have previously been used as a building block for polysaccharide-based polymers.[2] In particular, amylose, a linear polysaccharide consisting of α (1−4)-linked glucopyranose units, has been attracted growing interest in various fields.[3−5] Amylose adopts a coil or helical forms in aqueous solution.[6,7] Interestingly, amylose can form a rigid rod-like supramolecular complex by incorporating hydrophobic molecules into its left-handed helical cavity.[8−11] Furthermore, amylose can be synthesized in vitro using an enzymatic method.[12] α-Glucan phosphorylase is one of the most extensively studied enzymes used for the enzymatic polymerization of amylose.[13] Phosphorylase catalyzes the addition of a glucose unit from glucose monophosphate to the nonreducing end of a maltooligosaccharide (e.g., maltopentaose) in the absence of inorganic phosphate. Because this reaction is similar to a living polymerization, amyloses can be obtained with a low polydispersity. Furthermore, the degree of polymerization (D.P.) can be controlled by changing the ratio of a glycosyl donor/acceptor (the so-called primer).

In the past decade, amylose-based block or graft-type copolymers were synthesized enzymatically.[14−20] We have previously exploited this method to prepare polyethylene glycol (PEG)-*b*-amylose,[21,22] alkyl chain-*b*-amylose,[23,24] and amylose-*g*-cholesteryl poly(*l*-lysine).[25] Although there are many reports describing such classical polymers, very few reports have described amylose-based star copolymers, a type of spherical branched polymer.[26,27] Additionally, the effect of the star-shaped structure on their function (e.g., inclusion properties, gelation) are unknown. Star polymers generally have a smaller hydrodynamic volume, are well-defined, are multivalent, and have a three-dimensional structure compared with other polymers.[28−30] Furthermore, helical, amylose-based star polymers are expected to display co-operative (allosteric) properties as supramolecular multivalent hosts.

In the present study, we synthesized a series of amylose-based star polymer (1, 2, 4, and 8 arms) using a combination of click chemistry and phosphorylase-catalyzed polymerization and investigated the effect of their structures as multivalent hosts for hydrophobic compounds. We also prepared spermine-bearing amylose-based polymers and explored biofunctions of these polymers as nucleic acid chaperones.

Maltopentaose-based functional PEG derivatives were designed and synthesized as shown in Figure 1. The *N*-hydroxysuccinimide ester-modified PEG derivatives were initially functionalized with azide propyl amine. In the second step, maltopentaose-functionalized PEGs were prepared by

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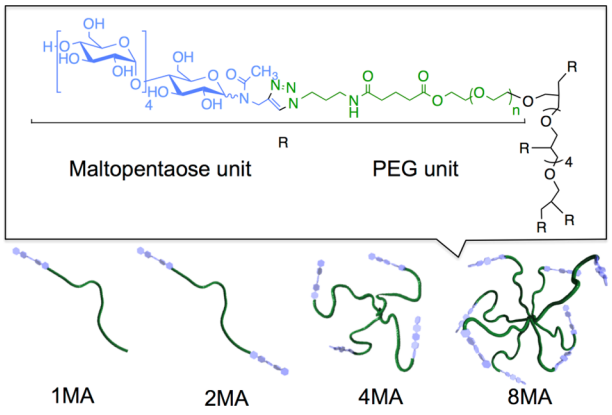

Figure 1. Chemical structures and illustrations of the maltopentaose functionalized PEG primers.

treating alkyne-functionalized maltopentaose[31] with appropriate azide-functionalized PEGs at room temperature for 24 h in *N*,*N*-dimethylformamide, followed by purification by dialysis to yield 1MA, 2MA, 4MA, and 8MA. The conversion rates for 1MA, 2MA, 4MA, and 8MA were 95, 93, 93, and 90%, respectively. The synthetic schemes and representative ^{1}H NMR spectra are given in Supporting Information.

Enzymatic polymerization of the maltopentaose-functionalized polymers was performed in a reaction catalyzed by phosphorylase b in bis−tris buffer at 40 °C (Figure 2a). The

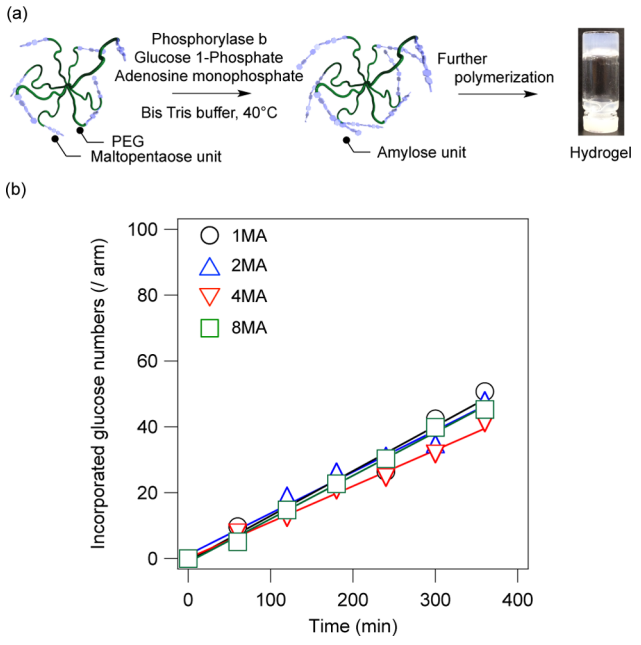

Figure 2. (a) Schematic illustration of the enzymatic polymerization and gelation of 8MA. (b) Time−course plot of the number of incorporated glucose molecules in multiarmed glyco polymers.

polymerization process was monitored by assaying inorganic phosphate.[32] Figure 2b shows the time−conversion plot for the primers with different molecular structures. The D.P. increased linearly with increasing reaction time, indicating the enzymatic polymerization was successful. Further polymerization yielded a viscous solution. For 8MA, a hydrogel was obtained because of effective cross-linking between the multiarmed molecules (Figures 2a and S1).[33,34] This represents a new in situ gelation system triggered by an enzymatic reaction. Further studies were performed using glyco copolymers with a D.P. of about 60 per arm, which is capable to form 10 turns; the helix was approximately 8 nm in length.

Formation of a complex with hydrophobic molecules is a well-known property of amylose as mentioned above. Amylose forms an inclusion complex with polyiodide ions (e.g., I_3^-) and the maximal absorption wavelength (λ_{max}) of the complex is related to the D.P. of amylose.[35] Therefore, we measured complexation of polyiodide ions with the polymers by UV−vis spectroscopy to obtain structural information for each polymer. The colorless polymer solutions immediately turned violet after adding a standard iodine−iodide solution. This is because polyiodide ions are encapsulated within the helical cavity of amylose. The λ_{max} values of the polyiodide ions−polymer complexes for 1 amylose arm polymer (1Amy), 2 amylose arm polymer (2Amy), 4 amylose arm polymer (4Amy), and 8 amylose arm polymer (8Amy) were 575, 574, 574, and 578 nm, respectively. The λ_{max} value of polyiodide ions−amylose (D.P. = ca. 60) complex, which was derived using Bank's equation ($(1/\lambda_{max}) = 1.558 \times 10^{-3} + 1.025 \times 10^{-2} \times (1/\text{D.P.})$), was 576 nm. The λ_{max} values for the polyiodide ions−polymer complexes were almost comparable to that of the polyiodide ions−amylose complexes, which suggests that the D.P. of the polymer is nearly equal to that of amylose. We therefore conclude that enzymatic polymerizations of 1MA−8MA are initiated from all of the potential sites on the polymer.[36] Because the maltopentaose moieties are located some distance from each other, this steric configuration is expected to be responsible for the accessibility of phosphorylase to all of the potential enzyme recognition sites.

Next, we investigated the effects of the structure on the ability of amylose-based polymers to encapsulate hydrophobic guest molecules (Figures 3 and S2−S16). The data revealed that relatively large elongated molecules (more than ca. 2 nm) are essential for the inclusion because the several relatively short, nonelongated molecules (e.g., pyrene, 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS), and 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS)) did not form a complex. Although the molecular sizes of diphenyl hexatriene (DPH), α-sexithiophene (6T), and curcumin are similar, only curcumin formed a complex with the polymers. These differences may be due to stabilization of the complex by formation of intermolecular hydrogen bonds between amylose and curcumin.[37] The polymers formed a 1:1 complex with bis pyrenyl propane (BPP), curcumin, and oligo phenylenevinylene (OPV) with binding constants that ranged from approximately 10^4 to 10^5 M^{-1} (per amylose unit). The circular dichroism (CD) spectra of the OPV/Glyco polymer complexes showed a positive Cotton effect (Figure S9). This result also indicates that these molecules were entrapped in the middle of the chiral helical cavity of amylose. However, compared with these systems, the stoichiometric ratios ([amylose]/[lipid]) for inclusion of C16SP, 14:0 LysoPC, and 1,2-dimyristoyl-*sn*- glycero-3-phosphocholine (DMPC) were 1:2, 1:4, and 1:4, respectively. In general, the alkyl chain moiety of the lipids is only entrapped by the amylose helix.[11] The NMR titration experiments revealed marked downfield shifts of the protons in the alkyl chain of C16SP (H_a), while much smaller shifts were observed for the aromatic ring (H_b) and spermine (H_c) protons (Figures 4a,b and S2). Our observations also indicate that the alkyl chain of C16SP was only covered by the amylose helix. Therefore, we believe that the entrapped lipids are confined to each end of the

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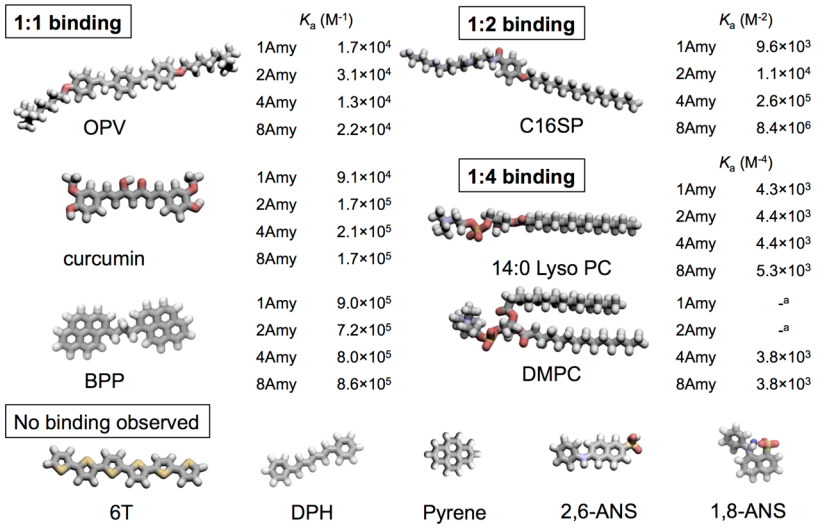
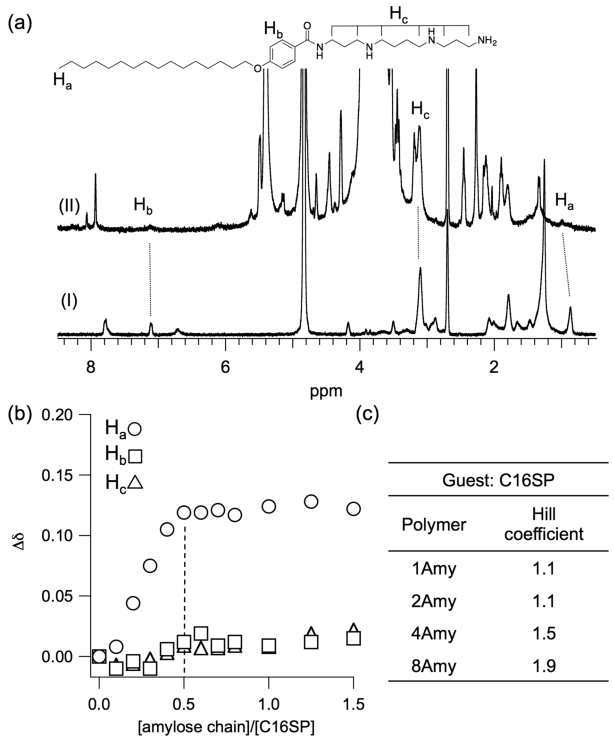


Figure 3. Chemical structures of the hydrophobic guest molecules. Binding constants were determined using Hill equation. Stoichiometry was determined from titration data using the mole ratio method. [a]No binding was observed.



Guest: C16SP	
Polymer	Hill coefficient
1Amy	1.1
2Amy	1.1
4Amy	1.5
8Amy	1.9

Figure 4. (a) ^{1}H NMR spectra of C16SP (0.69 mM) in a 95:5 (v/v) mixture of D_2O and DMSO-d_6 in the (I) absence and (II) presence of 8Amy (1.04 mM). (b) Changes in ^{1}H NMR chemical shifts for the functional regions H_a, H_b, and H_c of C16SP after the addition of 8Amy. (c) Hill coefficients for C16SP binding to the polymers.

helical segments of amylose (Figure S17) and that the polymers are multivalent host molecules.[38]

To gain further insight into the allosteric binding properties of the polymers, we analyzed the titration data using Hill's equation (Figure 3). The binding affinity for C16SP increased as the number of polymer arms increased. The binding affinity of the 8Amy for C16SP was about 10^3-fold greater than that of 1Amy for C16SP. In addition, 8Amy and 4Amy formed a complex with DMPC, whereas 1Amy and 2Amy did not bind to DMPC. Therefore, the binding of multivalent polymers to lipids is much more effective than that of 1Amy and 2Amy to lipids. Interestingly, the Hill coefficients also increased with increasing numbers of arms (Figure 4c), which might be due to a change in the structure of amylose from a random coil to a helix. Amylose normally has a random coil configuration and some parts of the helix are formed in an aqueous solution. Binding to the first lipid facilitates the conformational change of amylose to a helix. Binding to a lipid may also induce helix formation of neighboring amylose arms in highly branched polymers. This neighboring helix formation might explain the cooperative effects and might increase the polymer's binding affinity with increasing numbers of arms.[21,22,39,40] These observations indicate that, if multivalent molecules can be rationally designed, it may be possible to reinforce the polymer's binding affinity for lipids through conformational change of the host molecule. This is an attractive strategy for designing novel molecular host systems.

We also investigated the potential use of amylose-based star copolymers as artificial DNA chaperones. Several artificial DNA chaperones that catalyze DNA strand exchange have been reported.[41−44] These compounds have highly localized positive charges, which allow them to bind electrostatically to DNA strands and, therefore, concentrating DNA strands. Thus, we hypothesized that the amylose-based star copolymers bearing a cationic functional group would enhance DNA strand exchange. To test this hypothesis, we synthesized a series of spermine-functionalized hybrid polymers using a carbonyldiimidazole-mediated amide coupling reaction between the primary alcohol groups of amylose with spermine. The degrees of substitution of spermine were about 30 spermine residues per 100 glucose units of the polysaccharide (see Supporting Information).

Strand exchange kinetics were monitored using a Forster resonance energy transfer (FRET) assay with DNA duplexes labeled with FITC (fluorescein isothiocyanate) at the 3′ end of one strand and TAMRA (carboxyl tetramethyl rhodamine) at the 5′ end of the other strand (Figure 5a). Figure 5b shows the time-course of the strand exchange reaction of doubly labeled ds-DNA. The exchange reaction did not proceed in the absence of the polymers, and spermine alone did not mediate the strand exchange reaction. However, the strand exchange was

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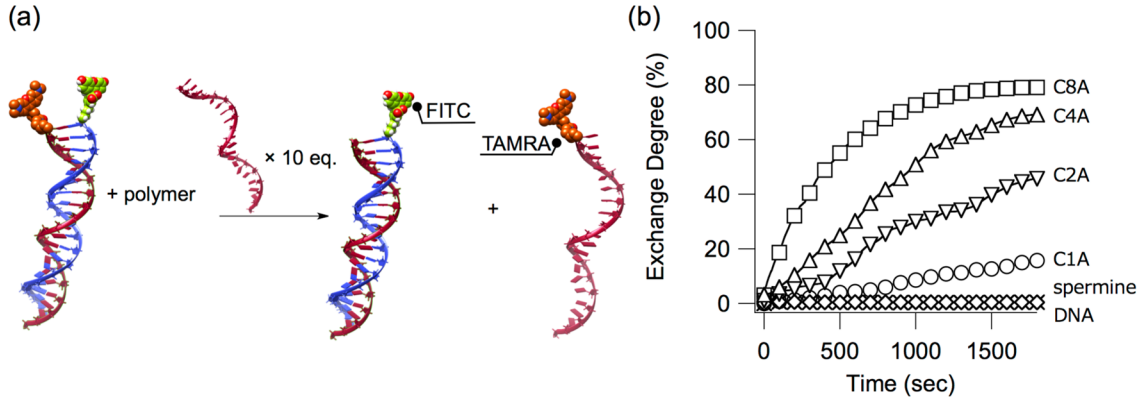

Figure 5. (a) Schematic illustration of the DNA strand exchange experiment. (b) Time-course of the DNA strand exchange reaction in the presence and absence of a series of cationic glyco polymers. C1A, C2A, C4A, and C8A denote cationic polymers with 1, 2, 4, and 8 amylose chains, respectively.

accelerated in the presence of the cationic polymer. The exchange ratio for the cationic polymer with eight amylose arms (C8A) was about 80% after 30 min. The apparent rates of the exchange reaction were determined by pseudo-first-order kinetic analysis (Table 1). C8A had the highest reaction rate constant of 1.2×10^{-4} s^{-1}, which was 44× greater than that of C1A. This strand exchange experiment clearly showed that the rate of the strand exchange increased as the number of amylose arms increased.

Table 1. Pseudo-First-Order Kinetic and Michaelis–Menten Parameters for the Cationic Polymers[a]

polymer	k' (s^{-1})	k_{cat} (s^{-1})	K_d (M)
C1A	2.8×10^{-6}	1.2×10^{4}	2.2×10^{-7}
C2A	2.7×10^{-5}	1.2×10^{4}	4.6×10^{-8}
C4A	4.8×10^{-5}	1.4×10^{4}	1.1×10^{-8}
C8A	1.2×10^{-4}	1.4×10^{4}	1.5×10^{-9}

[a] k': pseudo-first-order kinetic constant; k_{cat}: turn over number; K_d: dissociation constant.

To determine the effect of an increase in the number of polymer arms on the strand exchange rate, kinetic assays were performed using cationic polymers in which the initial rates were measured as the polymer concentration was increased. Because the fluorescence intensity is affected by changing the concentration of the substrate, we used a constant substrate concentration rather than a constant polymer concentration. The turn over number (k_{cat}) and dissociation constant (K_d) were determined for each polymer from the Lineweaver–Burk plots of $1/v_0$ versus 1/[spermine group] (Figure S18) and are presented in Table 1.

The k_{cat} values for all polymers remained almost constant. However, the k_{cat} values are independent of the arm number, the K_d values decreased with increasing numbers of arms. The K_d value C1A was 149 times larger than that of C8A. The lower K_d for C8A is probably due to an increase in the apparent cation concentration, which enhances substrate binding. This significant multivelent effect of star polymers in cationic environment induced an effective chaperone function. This result provide a guideline for rationally designing novel artificial chaperones for use in DNA strand exchanges.

In conclusion, we have designed and synthesized a series of polysaccharide-based star polymers with amylose arms. The 8-arm primer acted as an effective gelator when triggered enzymatically. Star polymers with a degree of polymerization of approximately 60 per arm served as allosteric multivalent supramolecular hosts. Furthermore, the cationic polymers displayed chaperone-like activity in DNA strand exchange reactions. We believe that these findings open a new research area for glyco biomaterials and will encourage researchers to examine their potential biomedical applications. Because of the multivalent properties of the star copolymers, this polymer may be useful as a biomedical material, especially in drug delivery systems. Research studies in this direction are now underway in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and additional experimental results. 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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