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Chemoenzymatic synthesis of amylose-grafted polyacetylenes

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Summary

This paper reports chemoenzymatic synthesis of amylose-grafted polyacetylenes according to the following reaction manners. Polymerization of a *N*-propargylamide monomer having a maltooligosacchairde chain was firstly carried out using a water-soluble Rh-catalyst in water, giving maltooligosaccharide-grafted polyacetylene. The ^{1}H NMR spectrum of the product supported the structure of the desired polyacetylene. Then, the enzymatic chain-elongation from the oligosaccharides of the polymer was performed using glucose-1-phosphate catalyzed by phosphorylase to give the polyacetylene having amylose side-chains. Furthermore, copolymerization of the monomer with the other *N*-propargylamide monomer was performed under the conditions similar to those of homopolymerization. The phosphorylase-catalyzed enzymatic chain-elongation of the copolymer was also carried out. The DLS measurement of the amylose-grafted polyacetylenes in alkaline solution was conducted.

Introduction

Researches concerning conjugated polymers, such as polyacetylene, polypyrrole, and polyphenylene, have attracted much attention in terms of the various practical applications of their interesting electrical and optical properties [1,2]. Moreover, interests and applications of the conjugated polymers have been extended to the biological fields. In order to endow the conjugated polymers with the biological functions derived from sugar residues, for example, the sugar-substituted conjugated polymers were synthesized by using the conjugated main-chain structures of polyaniline [3], polyisocyanide [4], polythiophene [5], polyphenylene [6,7], poly(*p*-phenylene ethynylene) [8], and poly(*p*-phenylene vinylene) [9].

In the series of these studies, we reported the synthesis of poly(*N*-propargylamide) with galactose residues [10,11]. It contained a *cis*-polyacetylene main-chain and was

obtained by the Rh-catalyzed polymerization of a corresponding *N*-propargylamide monomer. On the basis of the above study, the polyacetylenes having graft saccharide chains with higher degree of polymerization (DP), e.g., natural polysaccharides would be functional polymers with hybrid properties of polyacetylene and polysaccharide. Here we chose enzymatic chain elongation reaction in order to create the novel hybrid polymers, because the enzymatic chain-elongation reaction is a very powerful tool to obtain polysaccharides with well-defined structure [12,13]. It has been reported that the chemoenzymatic method is useful to produce the graft copolymers having the amylose side-chains of the natural polysaccharide, such as amylose-grafted polysiloxane [14-17], polystyrene [18,19], and poly(L-glutamic acid) [20]. To synthesize this type of the graft copolymers, maltooligosaccharide-grafted polymers were firstly prepared, and then, phosphorylase-catalyzed enzymatic chain-elongation using glucose-1-phophate (G-1-P) as monomer from non-reducing ends of the oligosaccharide side-chains on the polymers was performed, giving the amylose-grafted polymers. We also reported that this chemoenzymatic approach is applicable to the synthesis of an amylose-grafted chitosan [21].

In this paper, we report the application of the chemoenzymatic method to the synthesis of the novel amylose-grafted polyacetylenes (Scheme 1). A *N*-propargylamide monomer (**1**) having a maltooligosaccharide substituent was firstly prepared. Then, Rh-catalyzed polymerization of **1** was carried out to give the polyacetylene with pendant maltooligosaccharide chains. Finally, enzymatic chain-elongation of **2** using G-1-P was performed to produce the desired amylose-grafted polyacetylene **5**. The chain-elongation of the copolymer **4**, that was obtained by the copolymerization of **1** with the other *N*-propargylamide monomer **3**, was also conducted (Scheme 2). The dynamic light scattering (DLS) measurement was carried out to study the difference in the molecular size between the obtained amylose-grafted polyacetylenes and a pure amylose in aqueous solution.

Experimental

Materials

Maltoheptaose lactone was prepared from maltoheptaose according to the literature procedures [18]. Phosphorylase (300 u/mL) was supplied by Ezaki Glico Co. Ltd [22]. *N*-propargylethanamide (**3**) was prepared according to the literature [23]. Amylose was prepared by phosphorylase-catalyzed polymerization of G-1-P using maltoheptaose as a primer (G-1-P : primer = 100 : 1) in acetate buffer (pH = 6.2) at 40°C for 15 h. Other reagents and solvents were used as received.

*Synthesis of Monomer **1***

Under an atmosphere of argon, to a solution of maltoheptaose lactone (2.70 g, 2.34 mmol) in ethylene glycol (12.7 mL) was added propargylamine hydrochloride (1.07 g, 11.7 mmol) and triethylamine (1.63 mL, 11.7 mL) at room temperature. After the mixture was heated at 70°C for 18 h, it was poured into a large amount of acetone/methanol (3 : 1) to precipitate the crude product. The precipitate was isolated by filtration, washed with methanol, and dried under reduced pressure. After the

product was subjected by dialysis using cellulose ester membrane (molecular cut: 500), the obtained material was purified further by column chromatography on silica gel (eluent: methanol) to give **1** (0.560 g, 0.464 mmol) in 19.8 % yield.

^{1}H NMR (D_2O): δ 2.60 (HC≡C-, 1H), 3.43 – 4.04 (sugar protons except anomeric protons, -CH_2-C≡, 42H), 4.21 (C(C=O)CH(OH)C*H*(OH)-, 1H), 4.43 (C(C=O)C*H*(OH)-, 1H), 5.17 and 5.40 (anomeric protons, 6H).

*Typical Procedure for Polymerization of **1***

A typical polymerization procedure was as follows (entry 5, Table 1). Under an atmosphere of argon, a solution of catalyst, bis(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate ([Rh(cod)$_2$]BF_4) (0.00200 g, 0.00493 mmol) in water (0.20 mL) was added to a solution of **1** (0.0603 g, 0.0500 mmol) in water (0.20 mL) at 30°C and the mixture was stirred for 72 h at that temperature. The resulting solution was poured into a large amount of methanol to precipitate the polymeric product. The precipitate was isolated by filtration and dried under reduced pressure to give **2** (0.0243 g) in 40.3 % yield.

^{1}H NMR (D_2O): δ 3.34 – 3.96 (sugar protons except anomeric protons, -CH_2-C≡, 42H), 4.22 (C(C=O)CH(OH)C*H*(OH)-, 1H), 4.34 (C(C=O)C*H*(OH)-, 1H), 5.17 and 5.40 (anomeric protons, 6H), 5.80 – 6.40 (-CH=C-, 1H).

*Typical Procedure for Copolymerization of **1** with **3***

A typical copolymerization procedure was as follows (entry 3, Table 2). Under an atmosphere of argon, a solution of [Rh(cod)$_2$]BF_4 (0.00490 g, 0.0121 mmol) in water (0.50 mL) was added to a solution of **1** (0.0753 g, 0.0624 mmol) and **3** (0.0680 g, 0.0612 mmol) in water (0.50 mL) at 30°C. After the mixture was stirred at that temperature for 24 h, the resulting solution was poured into a large amount of methanol/ethanol (2 : 1) to precipitate the polymeric product. The precipitate was isolated by filtration and dried under reduced pressure to give **4** (0.0409 g) in 49.8 % yield.

^{1}H NMR (D_2O): δ 1.09 (CH_3), 2.23 (CH_2C=O), 3.39 – 4.00 (sugar protons except anomeric protons, -CH_2-C≡), 4.22 (C(C=O)CH(OH)C*H*(OH)-), 4.34 (C(C=O)C*H*(OH)-), 5.17 and 5.40 (anomeric protons), 6.15 (-CH=C-).

Enzymatic Chain-elongation

As a typical experimental procedure, **1** (0.0056 g, 0.0046 mmol, entry 5, Table 1) was dissolved in aqueous 0.20 mol/L acetate buffer (4.5 mL), and G-1-P (0.141 g, 0.460 mmol) was added to the solution. After the pH of the solution was adjusted to 6.2 by addition of 0.20 mol/L aqueous acetic acid, phosphorylase (ca. 12 units) was added, and the mixture was stirred for 16 h at 40°C. The precipitated product was isolated by centrifugation and dried by lyophilization to give **5** (0.0444 g).

Similarly, chain-elongation of **4** (0.0030 g, 0.0043 mmol, entry 4, Table 2) was carried out using G-1-P (0.134 g, 0.430 mmol) in the presence of phosphorylase (ca. 12 units)

in 0.20 mol/L acetate buffer (pH = 6.2). After the mixture was stirred for 18.5 h at 40°C, the precipitate was isolated by centrifugation and dried by lyophilization to give **6** (0.0630 g).

*Complex Formation **5** or **6** with Iodine*[19]

A typical procedure was as follows. A mixture of 1.0 mol/L potassium iodide solution (30 mL) and 0.0050 mol/L iodine solution (158 mL) was diluted with water to 250 mL (standard iodine-iodide solution). Polymer **5** or **6** (0.0030 g) was dissolved in DMSO (0.20 mL) and allowed to stand overnight. To the solution, the standard iodine-iodide solution (0.20 mL) was added, and the resulting solution was diluted with water (10 mL). The violet solution was characterized by UV-vis spectroscopy.

Measurements

NMR spectra were recorded on JEOL ECA 600 and ECX 400 spectrometers. Gel permeation chromatography (GPC) analyses were performed using a TOSOH 8012 with RI detector under the following conditions: Shodex GF-310HQ column with 0.1 mol/L $NaNO_3$ aqueous solution as the eluent at a flow rate of 0.5 mL/min at 60°C. The calibration curve was obtained using pullulan standards. The M_w determined by light scattering method was carried out using a multi angle light scattering (MALS) detector under the following conditions: OHpak SB803HQ column with 0.1 mol/L $NaNO_3$ aqueous solution as the eluent at a flow rate of 1.0 mL/min at 40°C. The M_n values were calculated from the obtained M_w and M_w/M_n values. UV-vis spectra were measured in a quartz cell (thickness 1 cm) at room temperature using a Jasco V-650 spectrometer. The DLS measurement was performed on a Zetasizer 3000 (Malvern Instruments).

Results and Discussion

*Polymerization of **1***

We already established that the sugar residue can be incorporated as the substituent of the *N*-propargylamide monomer by the reaction of sugar lactone with propargylamine without protection of sugar hydroxyl groups [10]. Thus we synthesized the monomer **1** from maltoheptaose lactone and propargylamine hydrochloride according to Scheme 3, and the structure of the isolated product was confirmed to be **1** by the ^{1}H NMR spectrum (Figure 1(a)).

We firstly performed the polymerization of **1** under the same conditions as those of the polymerization of the *N*-propargylamide having a galactose residue, i.e., in the presence of norbornadiene rhodium tetraphenylborate as the catalyst in a mixed solvent of THF and water, as reported in our previous papers [10,11]. However, the polymerization did not take place because of the formation of heterogeneous reaction mixture, which is due to the higher hydrophilicity of **1** in comparison with our previous monomer. To attempt the polymerization of **1** in homogeneous reaction system, a water soluble catalyst, $[Rh(cod)_2]BF_4$, was used (Scheme 1). Progress of the reaction was monitored by thin layer chromatography on silica gel (eluent; acetonitrile : water = 2 : 1) and the polymeric product was isolated as the fraction insoluble in

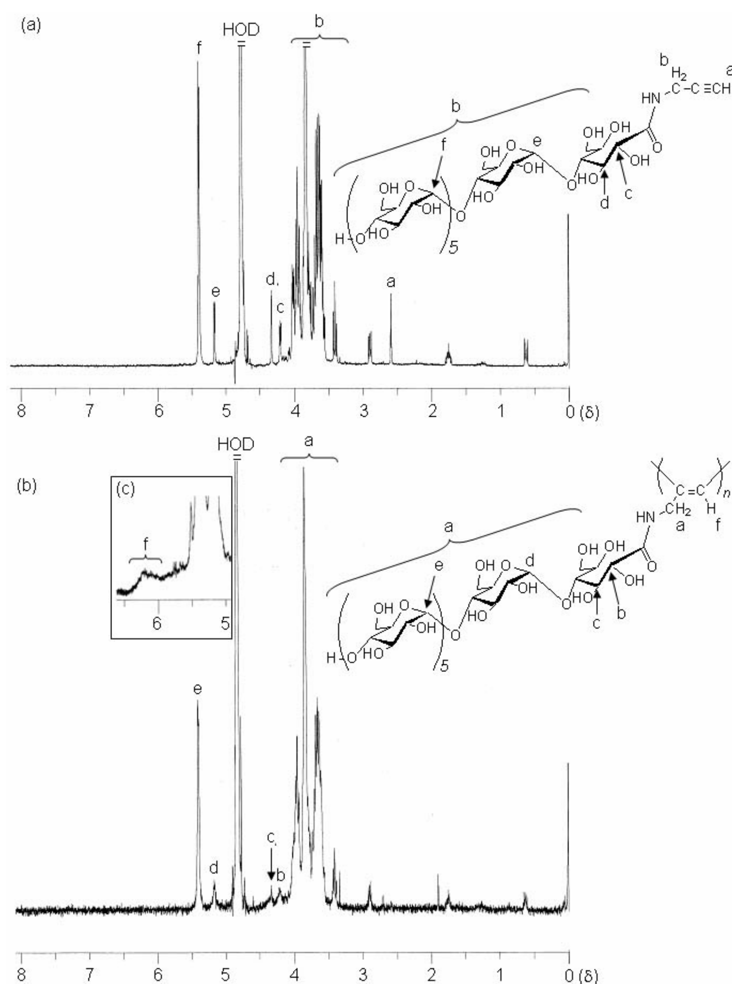
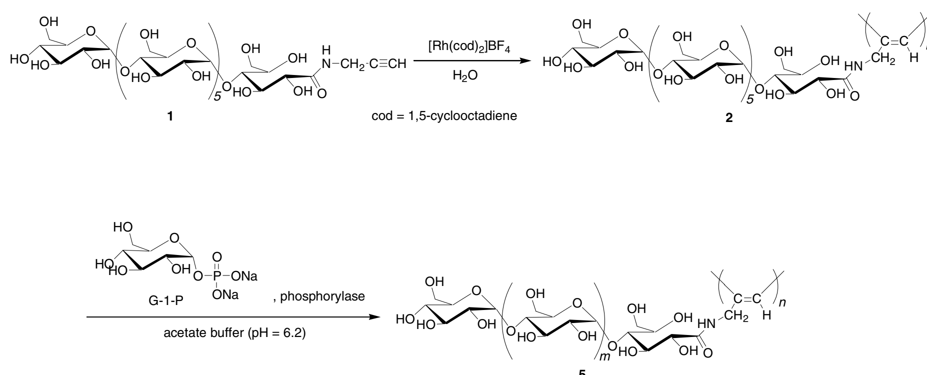


Figure 1. ^{1}H NMR spectra of **1** (a) and **2** (b; room temperature, 8 scans, c; 60°C, 800 scans) in D_2O.

methanol. The structure of the product was confirmed by the ^{1}H NMR measurement at room temperature (20ºC) and 60ºC. As shown in the ^{1}H NMR spectrum of the product at 20°C in D_2O (Figure 1(b)), the disappeared signal at δ 2.60 where the alkyne proton was observed before polymerization in Figure 1(a) and the broaden signals of sugar protons suggest that polyacetylene with sugar residues was formed as the final product. The signal due to the main-chain protons of the polyacetylene was not observed although the signal of the main chain protons should be appeared around at δ 6 in case of *cis*-conformation. When the ^{1}H NMR spectrum of the product was measured by the 800 times of repetitive measurement at a higher temperature of 60°C, the broad signal ascribable to the main-chain protons can be observed around at δ 5.8 – 6.4 as shown in Figure 1(c). Because the main-chain of the polyacetylene is shielded by the bulky oligosaccharide chains, the signal of the main-chain protons is probably difficult to be detected.



Scheme 1. Polymerization of **1** and enzymatic chain-elongation of **2**.

Table 1 shows the polymerization results of **1** under various conditions. The M_n values of all products estimated by GPC with 0.10 mol/L $NaNO_3$ aq. with RI detector using pullulan standards were around 3000. On the other hand, the M_n value of the product (entry 2 in Table 1) determined using MALS with the eluent same as above was 13900 with a polydispercity of 1.28. Since highly branched polymers have been known to take compact conformations [24], we assume that the actual molecular weight value is larger than that estimated using the calibration curve by pullulan standards and close to that determined using MALS. The highly grafted structures of the products with the pendant long oligosaccharide chains may cause underestimation of the M_n values by pullulan standards. Judging from the M_n value calculated based on the data by MALS, the average DP was estimated to be ca. 11.5. This data suggests that it is difficult to obtain the product with high DP by polymerization of **1** due to steric hindrance of the bulky oligosaccharide chain in each unit. The lower concentration of catalyst (2 mol%) and the lower reaction temperature (0°C) resulted in the lower yield of the product polymer (entries 1 – 2). The longer reaction times (4 to 72 h) as well as the higher reaction temperature (50°C) enhanced the yield of the product (entries 3 – 6).

Table 1. Polymerization of **1**[a]

Entry	[Catalyst]/[**1**] x 100 (mol %)	Temp. (°C)	Time (h)	Yield[b] (%)	$M_n(M_w/M_n)$[c]	$M_n(M_w/M_n)$[d]
1	2	30	4	4.0		
2	10	0	4	17.3	3430 (1.34)	13900 (1.28)
3	10	30	4	20.8	3540 (1.38)	
4	10	30	24	31.3	3190 (1.40)	
5	10	30	72	40.3	3330 (1.48)	
6	10	50	4	31.0	2790 (1.46)	

[a] Solvent: water, catalyst: $[Rh(cod)_2]BF_4$.
[b] Fraction insoluble in methanol.
[c] Determinedd by GPC with 0.10 mmol/L $NaNO_3$ aq. as the eluent using pullulan standards.
[d] Determined using MALS detector with 0.10 mmol/L $NaNO_3$ aq. as the eluent.

*Copolymerization of **1** with **3***

The copolymerizability of **1** was investigated by the copolymerization with the other *N*-propargylamide monomer **3** (Scheme 2). The reaction was performed in the various feed ratios of **1** to **3** under the similar conditions as those of the homopolymerization of **1** as described above. The polymeric products were isolated as the fractions insoluble in a mixed solvent of methanol and ethanol (2 : 1) except the product obtained by the feed ratio of **1** to **3** = 1 : 0.2 (entry 5, Table 2), which was isolated as the fraction insoluble in methanol. Figure 2 shows the ^{1}H NMR spectrum of the product (solvent: D_2O), which exhibits signals not only due to the sugar protons around at δ 3.3 – 5.4 but also due to the ethyl protons at δ 1.09 and 2.23. Unlike the ^{1}H NMR spectrum of **2** (Figure 1(b)), the signal assigned to the main-chain protons appears at δ 6.15 in this case. Appearance of this signal is probably attributed to existence of the ethanamide-substituted units (less hindered) in addition to the oligosaccharide-substituted units (more hindered). The ^{1}H NMR data fully support the copolymer structure **4** of the product. The unit ratio in the copolymer was calculated based on the ratio of the integrated signal intensity of the signal **a** to the signal **g**.

Table 2 shows the results of the copolymerization of **1** with **3**. The unit ratios of **1** in the copolymers increase with increasing molar ratios of **1** in feeds. In all cases,

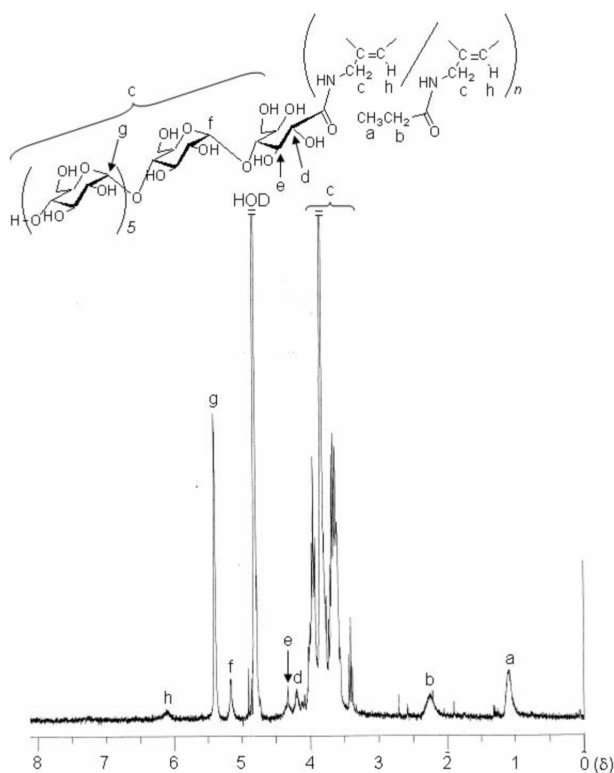
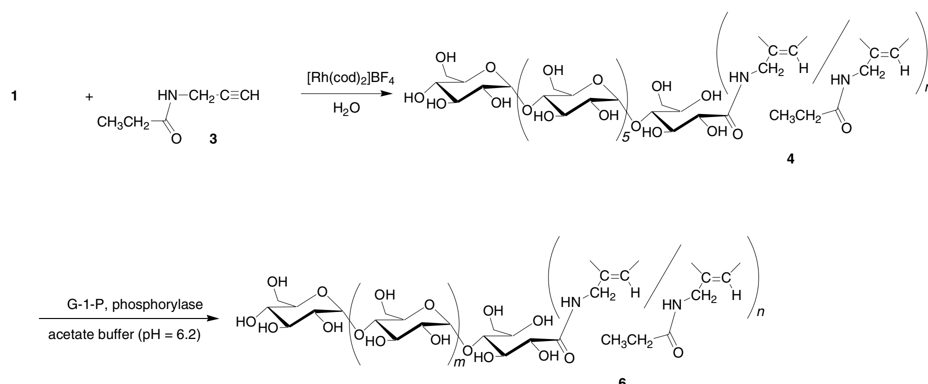


Figure 2. ^{1}H NMR spectrum of **4** in D_2O.



Scheme 2. Copolymerization of **1** with **3** and enzymatic chain-elongation of **4**.

however, the ratios of **1** in the copolymers are lower than those in the feeds. This is probably because the copolymers with higher contents of the units **1** are lost as the soluble fractions during the precipitation process. The M_n values determined by GPC with 0.10 mol/L $NaNO_3$ aq. as the eluent using pullulan standards are ca. 3100 – 3600, whereas the M_n value estimated using MALS (entry 3, Table 2) is 8870 (DP = ca. 9.7) with a polydispercity of 1.57. The difference between the above M_n and M_w values of **4** is due to the similar characteristic branched structure of **4** that was mentioned as the branched structure of **2**. The product yield was increased by extending the reaction time (entries 2 – 3).

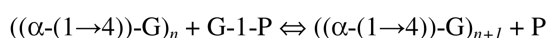
Table 2. Copolymerization of **1** with **3**[a]

Entry	Feed ratio	Time	Yield[b]	Unit ratio[c]	$M_n(M_w/M_n)$[d]	$M_n(M_w/M_n)$[e]
	1 : **3**	(h)	(%)	**1** : **3**		
1	1 : 2	4	21.2	1 : 2.43	3270 (1.56)	
2	1 : 1	4	23.6	1 : 1.32	3630 (1.51)	
3	1 : 1	24	49.8	1 : 1.25	3180 (1.62)	8870 (1.57)
4	1 : 0.5	4	39.2	1 : 0.86	3410 (1.57)	
5	1 : 0.2	4	23.2[f]	1 : 0.36	3320 (1.65)	

[a] Solvent: water, catalyst: $[Rh(cod)_2]BF_4$ (10 mol% for **1** + **3**), reaction temperature: 30°C.
[b] Fraction insoluble in a mixed solvent of methanol and ethanol (2 : 1).
[c] Determined by ^{1}H NMR spectra.
[d] Determined by GPC with 0.10 mmol/L $NaNO_3$ aq. as the eluent using pullulan standards.
[e] Determined using MALS detector with 0.10 mmol/L $NaNO_3$ aq. as the eluent.
[f] Fraction insoluble in methanol.

*Enzymatic Chain-elongation of **2** and **5** Giving Amylose-grafted Polyacetylenes*

It has been well known that the phosphorylase-catalyzed chain-elongation using G-1-P as monomer is initiated from a maltooligosaccharide [25,26]. The propagation proceeds by the following reversible reaction to form a α-(1→4)-glucan chain, i.e., amylose.



$$((\alpha\text{-}(1\rightarrow4))\text{-G})_n + \text{G-1-P} \Leftrightarrow ((\alpha\text{-}(1\rightarrow4))\text{-G})_{n+1} + \text{P}$$

In the reaction, a glucose unit is transferred from G-1-P to the non-reducing 4-OH terminus of a α-(1→4)-glucan chain, which results in release of inorganic phosphate (P). At the beginning of the reaction, a maltooligosaccharide as a primer is required.

The enzymatic chain-elongation from **2** (entry 5 in Table 1) or **4** (entry 4 in Table 2) was performed using 100 equivalents of G-1-P for the primer chain catalyzed by phosphorylase in acetate buffer (Schemes 1 and 2). The products were gradually precipitated from the solution with the progress of the reaction. The precipitates were isolated by centrifugation and lyophilized to give amylose-grafted polyacetylenes. The solubility of the products was same as that of amylose, i.e., soluble in DMSO and alkaline aqueous solution, but insoluble in other typical organic solvents and water. Figure 3 shows the ^{1}H NMR spectrum of the product from **2** in 1 mol/L NaOD-D_2O. The significant difference in the signal patterns of the spectra between before (Figure 1 (b)) and after (Figure 3) the chain elongation reaction is no appearance of the signal due to the H1 proton of a glucose residue closest to the main-chain. This indicates progress of the enzymatic chain-elongation, leading to the formation of saccharide chains with high DP, i.e., amylose. The ^{1}H NMR spectrum of the product from **4** was almost same to that of Figure 3, indicating production of **6**. Because of no signals due to the main-chain protons in the ^{1}H NMR spectrum of **5** or **6** as shown in Figure 3, the spectroscopic pattern is quite similar to that of amylose. Difference between these products (branched polymers) from amylose was revealed by the DLS measurement in alkaline solution (vide infra).

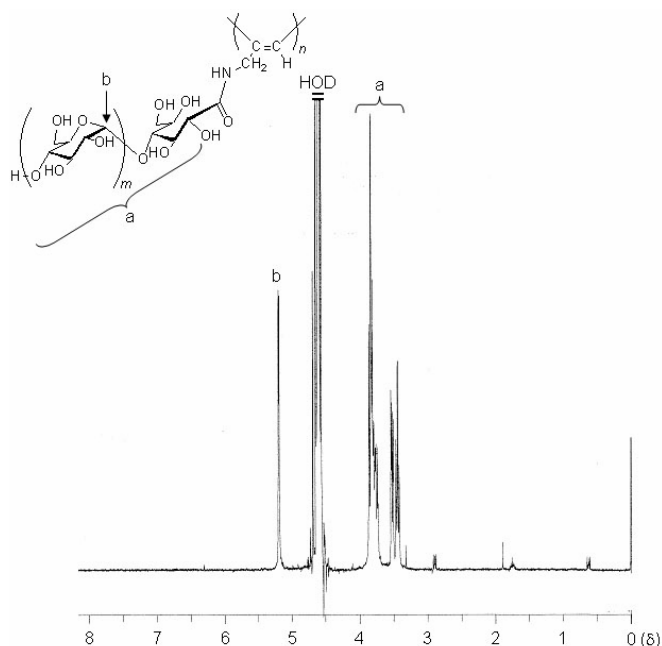
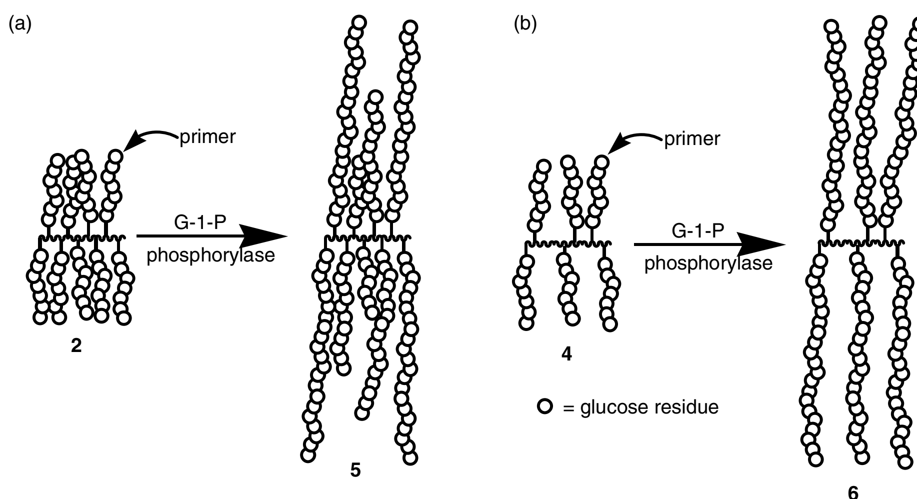


Figure 3. ^{1}H NMR spectrum of **5** in 1 mol/L NaOD-D_2O.

The complex formation with iodine is a well-known characteristic property of amylose [27]. The colorless solutions of **5** and **6** in DMSO turned to violet after the

addition of a standard iodine-iodide solution to the polymer solution, as the same color change in the complex formation of amylose with iodine. Values of the λ_{max} of UV-Vis spectra of the iodine complexes with **5**, **6**, and amylose were 577, 586.5, and 586.5 nm. These data indicated that the average DP of **6** was probably comparable to that of amylose, whereas the average DP of **5** might be lower in comparison with amylose [18]. On the basis of the above data, we assume the following scheme explaining the difference in the enzymatic chain-elongation using **2** and **5**. The enzymatic chain-elongation was hardly initiated from all the potential sites of the primers (maltooligosaccharides) on **5** due to steric hindrance, resulting in the lower average DP (Scheme 3(a)). On the other hand, the less hindered orientation of the primers on **6** due to existence of the units **3** in the main-chain was probably more suitable for initiation from the most of the potential sites for the chain elongation reaction (Scheme 3(b)). Therefore, the average DP of **6** became same as that of amylose.



Scheme 3. Proposed schematic image for enzymatic chain-elongation of **2** (a) and **4** (b).

The DLS measurement was conducted to reveal the difference in the molecular size among the branched polyacetylenes (**5** and **6**) and amylose in solution. The DLS data (Figure 4) of amylose in 1.0 mol/L NaOH solution (1.0 mg/mL) shows two mean diameters of 13.2 and 324 nm. The former is attributable to the size of single molecule in solution and the latter is probably due to the molecular aggregates. Formation of the large aggregates from amylose in alkaline solution has been reported in previous article [28]. The two mean diameters of 21 and 324 nm ascribable to the sizes of single polymer molecule and molecular aggregates, respectively, are observed in the DLS measurement of **5** in the alkaline solution. The larger diameter of a molecule **5** obtained by the DLS analysis compared with that of an amylose molecule is reasonably explained by the molecular structure having several amylose graft chains in a polymer, although the average DP of amylose chains on **5** is smaller than that of a sole amylose. A frequency of the molecular aggregates (30 %) formed from **5** was lower than that from amylose (56 %). This is probably because that intramolecular aggregation of amylose graft chains in **5** is more predominant than their

intermolecular aggregation. In fact, a mean diameter of 31 nm, larger than that from amylose or **5**, to the exclusion of the molecular aggregates appears in the DLS result of **6**, because **6** has amylose graft chains with the higher average DP than that in **5**.

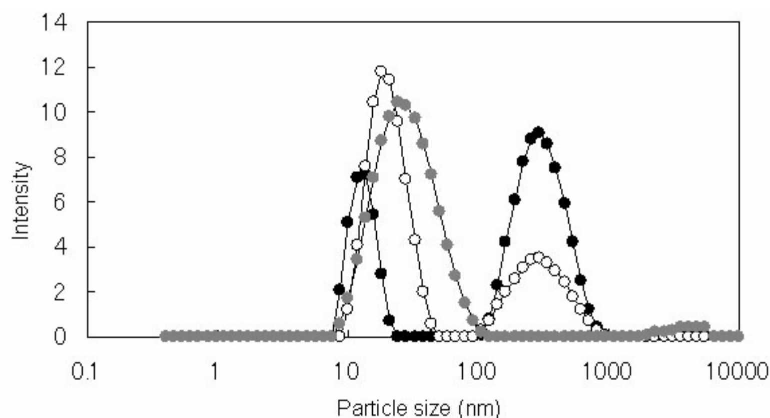


Figure 4. DLS profiles of amylose (●), **5** (○), and **6** (●) in 1.0 mol/L NaOH aqueous solution (sample concentration; 1.0 mg/L).

Conclusion

In this study, we have synthesized amylose-grafted polyacetylenes by chemo-enzymatic method. Rh-catalyzed polymerization of **1** having a maltooligosaccharide chain was firstly carried out in water. The structure **2** of the product was confirmed by the ^{1}H NMR spectrum and the M_w value determined using MALS was 13900. The copolymerization of **1** with **3** took place under the conditions similar to those of homopolymerization, giving **4**. Then, the phosphorylase-catalyzed enzymatic chain-elongation of **2** and **4** was performed using G-1-P to give amylose-grafted polyacetylenes **5** and **6**, respectively. The results for complex formation of **5** and **6** with iodine suggested that chain-elongation was hardly initiated from all the potential sites of the primers on **5** due to steric hindrance, whereas chain elongation reaction from the most of the potential sites on **6** proceeded because of their less steric hindrance. The DLS measurement of **5** and **6** in NaOH aq. indicated a larger molecular size of **6** in solution than that of **5**.

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