Polymer Bulletin 49, 405–410 (2003)

Polymer Bulletin

Glycoconjugated polymer 6. Synthesis of poly[styrene-*block*-(styrene-*graft*-amylose)] via potato phosphorylase-catalyzed polymerization

Atsushi Narumi[1], Kosei Kawasaki[2], Harumi Kaga[2], Toshifumi Satoh[1], Naoya Sugimoto[1], Toyoji Kakuchi[1] (✉)

[1]Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo, 060-8628, Japan
[2]National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan
E-mail: kakuchi@poly-mc.eng.hokudai.ac.jp, Tel(Fax): 81-11-706-6602

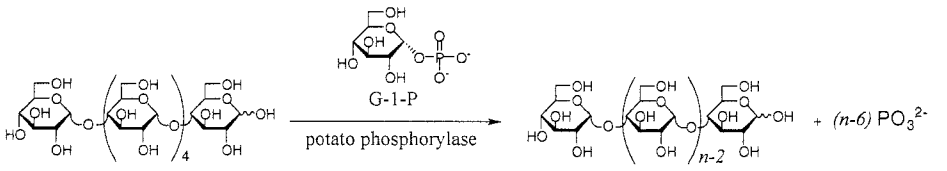
Received: 29 November 2002/Accepted: 22 December 2002

Summary

The potato phosphorylase-catalyzed polymerization of α-D-glucose-1-phosphate (G-1-P) onto poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)] (**1**) was performed at the molar ratios of $[G\text{-}1\text{-}P]_0$ and $[maltohexaose]_0$ of 35, 80, and 250. The product was found to be soluble in dimethyl sulfoxide, which was a good solvent for amylose, and showed the complex-formation with iodine, indicating that the product was assignable to poly[styrene-*block*-(styrene-*graft*-amylose)] (**2**). The quantitative analysis of the liberated phosphoric acid gave the average degree of polymerization of the glucose unit (*n*) as 27, 51, and 180 for **2-I**, **2-II**, and **2-III**, respectively.

Introduction

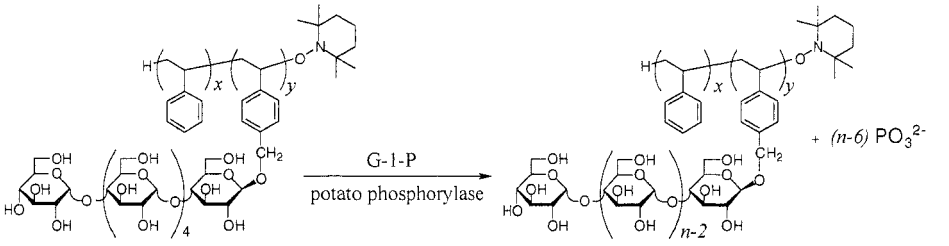
Potato phosphorylase catalyzes the polymerization of glucose-1-phosphate (G-1-P) to afford a polysaccharide consisting of α-1,4 linked glucose units, i.e., amylose. In general, this enzymatic reaction requires α-1,4 linked linear oligosaccharides, e.g., maltopentaose, maltohexaose, maltoheptaose, and dextrin, namely the primer (Scheme 1). After the pioneering work by Ziegast and Pfannemüller [1], several research groups reported that synthetic polymers and/or silica gel, which were covalently bonded with the primers, were prepared and modified into amylose-based materials by the potato phosphorylase-catalyzed polymerization [2-10].



Scheme 1. Potato phosphorylase-catalyzed polymerization of G-1-P onto maltohexaose to afford amylose.

Recently, there has been noteworthy progress in the precise synthetic methodology for the polymerization of a vinyl saccharide [11-20]. Most of the reports described the polymerization of a vinyl monomer having monosccharides, while we reported that the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-mediated radical polymerization [21-23] was successfully applicable to the polymerization of 4-vinylbenzyl maltohexaoside peracetate to give the maltohexaose-conjugated polymer [24,25]. Thus, our system combined with the subsequent enzymatic polymerization has the potential to become a useful method to prepare a variety of architectures composed of polystyrene and amylose.

We report here the potato phosphorylase-catalyzed polymerization of G-1-P onto poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)] (**1**) to afford a novel type of architecture such as poly[styrene-*block*-(styrene-*graft*-amylose)] (**2**), as shown in Scheme 2.



1 (*x* = 90, *y* = 6) **2** (*x* = 90, *y* = 6)

Scheme 2. Potato phosphorylase-catalyzed polymerization of G-1-P onto poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)] (**1**) to afford poly[styrene-*block*-(styrene-*graft*-amylose)] (**2**).

Experimental

Materials

Potato phosphorylase (EC 2.4.1.1) was purified according to the method reported by Kamogawa et al. [26] and Schneider et al. [27]. The activity of the potato phosphorylase was determined by the method of Kamogawa et al. [26], except that the assay of the liberated phosphoric acid was performed by the method of Parvin and Smith [28]. A seamless cellulose tube (UC24-32-100) was obtained from the Viskase Sales Co. The α-D-Glucose-1-phosphate dipotassium salt hydrate (Sigma, 99 %), dimethyl sulfoxide (DMSO) (Kanto Chemical Co., >99.0 %), spectroscopy grade DMSO (Kanto Chemical Co., >99.7 %), 1 N potassium iodide solution (Kishida Chemical Co.), 0.01 N iodine solution (Kishida Chemical Co.), and all other chemicals were used without further purification.

Instruments

The ^{1}H and ^{13}C NMR spectra were recorded using a Bruker ASX300 NMR spectrometer. The optical rotations were measured using a Jasco DIP-1000 digital polarimeter. The ultraviolet-visible (UV-vis) spectra were measured at 23 °C with 5 mm path lengths using a Jasco V-550 spectrophotometer.

Enzymatic polymerization

An example of the procedure is described for the preparation of **2-I**. Poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)], **1**, (0.12 g, 7.5 μmol, $[\alpha]_D$ = +60) and G-1-P (0.52 g, 1.5 mmol) were dissolved in a mixture of 0.1 M maleic acid buffer (pH = 7.5, 19 mL) and DMSO (5.0 mL). The mixture was preincubated overnight with stirring at 45 °C. To the mixture was added a solution containing potato phosphorylase (2.5 unit/mL, 2.1 mL) every 24 h followed by incubating at 37 °C. The consumption of G-1-P was monitored by the assay of liberated phosphoric acid during the reaction. After incubation for 96 h, the solution was heated at 95 °C for 5 min to deactivate the enzyme, cooled, and then filtered. The filtrate was poured into ethanol (60 mL) and allowed to stand overnight. The supernatant was removed by decantation and then the precipitate was transferred to a cellulose tube and dialyzed for 2 days with distilled water. The aqueous suspension was evaporated, and then poured into ethanol (ca. 50 mL). The precipitate was washed with ethanol and diethyl ether and dried *in vacuo* to give poly[styrene-*block*-(styrene-*graft*-amylose)], **2-I**, as a white solid. Yield: 0.22 g. $[\alpha]_D$ = +113° (*c* 0.5, DMSO). Figure 1 shows the ^{1}H NMR and ^{13}C NMR spectra of **2-I**. The yield of the liberated phosphoric acid (60 %) indicated that the average degree of the polymerization of amylose (*n*) and the weight average molecular weight for **2-I** were 27 and 36,800, respectively.

Complex Formation with Iodine

The typical procedure was as follows: A mixture of 1 N potassium iodide solution (30 mL) and 0.01 N iodine solution (158 mL) was diluted with water to 250 mL (standard iodine-iodide solution). Polymer **2-I** (3.0 mg) was added to dimethyl sulfoxide (0.20 mL) then 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. UV-vis (5 mm cell): λ_{max} (abs) = 595 nm (0.94).

Results and discussion

Enzymatic polymerization onto 1

Poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)], **1**, which was prepared by the two-step nitroxide-mediated polymerizations and subsequent deacetylation, showed a high hydrophilic property as expected from the multiple hydroxyl groups due to maltohexaose [24]. The average degree of the polymerization of the styrene unit (*x*) and that of the 4-vinylbenzyl maltohexaoside unit (*y*) in **1** is 90 and 6, respectively. Hence, **1** should act as a hexa-functional primer for the enzymatic polymerization.

The polymerization of the α-D-glucose-1-phosphate dipotassium hydrate (G-1-P) onto poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)] (**1**) was performed in a mixture of maleic acid buffer and dimethyl sulfoxide (DMSO), as shown in Scheme 2. The concentrations of G-1-P in the feeds ($[G\text{-}1\text{-}P]_0$) were constant at 63 mmol·L^{-1}.

The polymerizations were performed at the molar ratio of $[G\text{-}1\text{-}P]_0$ and $[\text{maltohexaose}]_0$ (F, i.e., $[\text{maltohexaose}]_0 = [\mathbf{1}]_0/6$) of 35, 80, and 250 to afford products **2-I** ~ **2-III**. As a control, the same polymerization onto maltohexaose (**3**) was carried out to produce amylose (**4**), as illustrated in Scheme 1. The consumption

of G-1-P was monitored by assay of the liberated phosphoric acid during the enzymatic polymerization. It was found that the polymerization onto **1** required a large amount of enzyme and also a long time compared to that onto **3**. A possible reason for this result is described in the following section. After the consumption of G-1-P reached ca. 60 %, the system using **1** was quenched to afford the product (**2**) as a white powder. Table 1 shows the results of the polymerization and characterization of the products.

Table 1. Phosphorylase-catalyzed polymerization of G-1-P[a] onto **1** and **3**

Starting material		Product				Iodine-complex[g]	
	F[d]	Sample	Conversion[e] (%)	n[e]	$[\alpha]_D$[f] (deg)	λ_{max} (nm)	Abs.
1[b]	35	**2-I**	60	27	+113	595	0.94
	80	**2-II**	56	51	+139	598	0.92
	250	**2-III**	68	180	+160	605	0.95
3[c]	35	**4-I**	78	33	+156	564	0.80
	80	**4-II**	73	64	+168	578	0.92
	250	**4-III**	79	198	+185	589	0.99

[a] $[G\text{-}1\text{-}P]_0 = 63$ mmol·L^{-1}; [b] [phosphorylase]/$[G\text{-}1\text{-}P]_0 = 14/1$ unit·mmol^{-1}, time = 96 h; [c] [phosphorylase]/$[G\text{-}1\text{-}P]_0 = 3.5/1$ unit·mmol^{-1}, time = 24 h; [d] ratio of $[G\text{-}1\text{-}P]_0$ and $[\text{maltohexaose}]_0$; [e] determined by the liberated phosphoric acid; [f] measured in DMSO, c 0.5; [g] sample, 0.30 g·L^{-1}; KI, 0.24 mol·L^{-1}; iodine, 12.6 mmol·L^{-1}; DMSO, 20 mg·L^{-1}.

Although **1** showed high hydrophilic property, **2** was soluble only in DMSO and insoluble in water leading to a precipitate. Figure 1 shows the ^{1}H NMR and ^{13}C NMR spectra of **2-I** in DMSO-d_6. The signals due to the aromatic moiety appeared at 6.2 ~ 7.4 ppm along with the characteristic sharp signals due to amylose at 4.73 (OH-6), 5.09 (H-1), 5.45 (OH-2), 5.57 ppm (OH-3) in the ^{1}H NMR of the product. Similarly, the signal due to the aromatic moiety appeared at 124 ~ 130 ppm along with the six signals due to the amylose at 61.3 (C-6), 72.4 (C-5), 72.7 (C-2), 74.1 (C-3), 79.6 (C-4), 100.9 ppm (C-1) in the ^{13}C-NMR of the product.

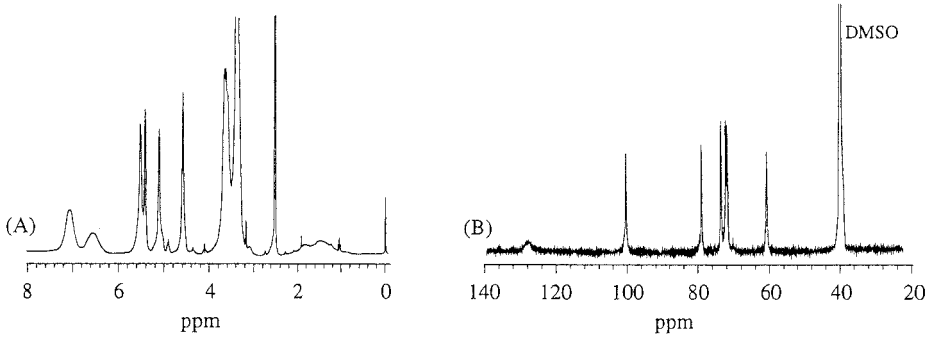


Figure 1. ^{1}H NMR spectrum (A) and ^{13}C NMR spectrum (B) of **2-I** in DMSO-d_6.

The complex-formation with iodine is a well-known characteristic property of amylose [29]. It was found that the clear solution of **2** in DMSO immediately turned to violet after the addition of a standard iodine-iodide solution. Table 1 shows the maximum wavelength (λ_{max}) and absorbance (abs) of the maximum absorption for the violet solution, which was characterized by UV-vis spectroscopy. These results

indicated that **2** was assignable to poly[styrene-*block*-(styrene-*graft*-amylose)].
In general, the assay of the liberated phosphoric acid provides the average degree of polymerization of the glucose unit (*n*) for a product in the potato phosphorylase-catalyzed polymerization [1-10]. The assay gave the *n* values of 27, 51, and 180 for **2-I**, **2-II**, and **2-III**, respectively. The isolated yields of **2** were in good agreement with the theoretical one based on the *n* values. As one example, the polymerization onto 0.12 g of **1** afforded 0.22 g of **2-I**, while the theoretical yield was 0.27 g. Similarly, the analysis of the liberated phosphoric acid gave the *n* values of 33, 64, and 198 for **4-I**, **4-II**, and **4-III**, respectively. The λ_{max} of the idodine-complex with **4** increased from 563 nm to 589 nm with the increasing *n* values from 33 to 198, as expected from the results in the literature [26]. However, the λ_{max} of the idodine-complex with **2-I** ~ **2-III** showed nearly constant values in the raged of 595 ~ 605 nm. The idodine-complex with **2** might be promoted through the side-by-side association of helices by intermolecular aggregation of amylose chains.
Although we have no data about the polydispersity for **2**, it was suggested by Kobayashi et al. [3] that the enzymatic polymerization was hardly initiated from all the potential sites of the multi-functional primer due to steric hindrance. Furthermore, the dynamic laser light scattering measurement indicated that **1** was stably suspended in water by forming micelle-like aggregates consisting of a hydrophilic maltohexaose shell and a hydrophobic polystyrene core [24]. Although the potential sites in **1** should be crowded, the enzymatic polymerization of G-1-P onto **1** successfully proceeded. Therefore, the procedure described in the paper should be one direct method to afford a novel type of architecture such as poly[styrene-*block*-(styrene-*graft*-amylose)].

Conclusions

The potato phosphorylase-catalyzed polymerization of G-1-P onto poly[styrene-*block*-(4-vinylbenzyl maltohexaoside)] successfully proceeded to afford poly[styrene-*block*-(styrene-*graft*-amylose)]. Hence, the precise synthesis of the maltohexaose-conjugated polymer combined with subsequent enzymatic polymerization should have the potential to become a useful method to prepare a variety of architectures composed of polystyrene and amylose.

Acknowledgements. The author, A.N., would like to thank the Japan Society for the Promotion of Science for their financial support of this research.

References

1. Ziegast G, Pfannemüller B (1987) Carbohydr Res 160:185.
2. Braunmühl V, Jonas G, Stadler R (1995) Macromolecules 28:17.
3. Kobayashi K, Kamiya S, Enomoto N (1996) Macromolecules 29:8670.
4. Enomoto N, Furukawa S, Ogasawara Y, Akano H, Kawamura Y, Yashima E, Okamoto Y (1996) Anal Chem 68:2798.
5. Loos K, Stadler R (1997) Macromolecules 30:7641.
6. Loos K, Stadler R (1997) Macromol Rapid Commun 18:927.
7. Kamiya S, Kobayashi K (1998) Macromol Chem Phys 199:1589.
8. Akiyoshi K, Kohara M, Ito K, Kitamura S, Sunamoto J (1999) Macromol Rapid Commun 20:112.

9. Haupt M, Knaus S, Rohr T, Gruber H (2000) J Macromol Sci, Pure Appl Chem A37:323.
10. Loos K, Müller AHE (2002) Biomacromolecules 3:368.
11. Yamada K, Yamaoka K, Minoda M, Miyamoto T (1997) J Polym Sci, Part A: Polym Chem 35:255.
12. Yamada K, Minoda M, Miyamoto T (1997) J Polym Sci, Part A: Polym Chem 35:751.
13. Yamada K, Minoda M, Miyamoto T (1999) Macromolecules 32:3553.
14. Yamada K, Minoda M, Fukuda T, Miyamoto T (2000) J Polym Sci, Part A: Polym Chem 39:459.
15. Loykulnant S, Hayashi M, Hirao A (1998) Macromolecules 31:9121.
16. Ohno K, Tsuji Y, Fukuda T (1998) J Polym Sci, Part A: Polym Chem 36:2473.
17. Ohno K, Izu Y, Yamamoto S, Miyamoto T, Fukuda T (1999) Macromol Chem Phys 200:1619.
18. Li ZC, Liang YZ, Li FM (1999) Chem Commun 16:1557.
19. Yongming C, Wulff G (2001) Macromol Chem Phys 202:3273.
20. Narumi A, Matsuda T, Kaga H, Satoh T, Kakuchi T (2002) Polymer 43:4835.
21. Georges MK, Vergin RPN, Kazmaier PM, Hamer GK (1994) Trends Polym Sci 2:66.
22. Hawker CJ (1997) Acc Chem Res 30:373.
23. Hawker CJ, Bosman AW, Harth E (2001) Chem Rev 101:3661.
24. Narumi A, Matsuda T, Kaga H, Satoh T, Kakuchi T (2001) Polymer J 33:939.
25. Narumi A, Satoh T, Kaga H, Kakuchi T (2002) Macromolecules 35:699.
26. Kamogawa A, Fukui T, Nikuni Z (1968) J Biochem 63:361.
27. Schneider EM, Becker JU, Volkmann, D (1981) Planta 151:124.
28. Parvin R, Smith RA (1969) Anal Biochem 27:65.
29. Banks W, Greenwood CT, Khan KM (1971) Carbohydr Res 17:25.