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Chemoenzymatic synthesis of amylose-grafted alginate and its formation of enzymatic disintegratable beads

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ABSTRACT

This paper reports the chemoenzymatic synthesis of an amylose-grafted alginate. An amine-functionalized maltooligosaccharide was chemically introduced into sodium alginate by condensation with the carboxylates of the alginate to produce a maltooligosaccharide-grafted alginate. Then, a phosphorylase-catalyzed enzymatic polymerization of glucose 1-phosphate from the graft chain ends on the alginate derivative was performed, giving an amylose-grafted alginate. Beads were produced from the product by treatment with $CaCl_2$ aqueous solution, which exhibited disintegratable property by β-amylase. Furthermore, the effect of the enzymatic reaction on the release of an entrapped dye compound from inside of the beads was examined.

1. Introduction

Natural polysaccharides are important for *in vivo* functions such as providing an energy source and as a structural material (Berg, Tymoczko, & Stryer, 2006). Because the polysaccharides are naturally recycled carbon resources and considered to be eco-friendly due to their biodegradability, it is expected that the use of the polysaccharides will lead to production of environmentally benign materials. Alginic acid is one of the most abundant natural polysaccharides. It consist of (1→4) linked β-D-mannuronic acid and α-L-guluronic acid residues of widely varying composition and sequence as shown in Scheme 3 (Schuerch, 1986). It occurs both as a structural component in marine brown algae (*Phaeophyceae*), comprising up to 40% of the dry matter, and as capsular saccharides in soil bacteria. Due to the presence of the carboxylic acid groups in the saccharide residues, alginic acid has an anionic nature, forming alginate salts with cationic metals such as Ca^{2+} and Na^{+}. Calcium alginate is insoluble and though swells in water, whereas sodium alginate is soluble in water. Accordingly, alginate beads were conventionally prepared by gelation of sodium alginate aqueous solution by adding $CaCl_2$ aqueous solution (Prasad & Kadokawa, 2009). The alginate hydrogel beads have been extensively used for last several decades for applications such as drug release and immobilization of enzymes.

Besides the abundant natural polysaccharides, branched polysaccharides composed of the multiple unit structures are often found in nature. For example, arabinoxylan, gum arabic, and guaran play important roles in moisture maintenance and protection against bacteria (Schuerch, 1986). These materials are composed of two or more different kinds of saccharide components, which contribute to their prominent functions. Therefore, the preparation of branched or grafted artificial heteropolysaccharides using the abundant polysaccharide resources is a promising topic in the research field of bio-based materials. Although chemical synthesis of polysaccharides is necessarily performed by the multiple reaction steps including protection and deprotection of hydroxyl groups due to their complicated structures, enzymatic polymerization has recently been developed as a facile and useful tool for the preparation of precisely regio- and stereocontrolled polysaccharides under mild conditions (Kobayashi, 2007; Kobayashi, Ohmae, Fujikawa, & Ochiai, 2005; Kobayashi, Uyama, & Kimura, 2001; Shoda, Izumi, & Fujita, 2003). For enzymatic polymerization, substrates can be employed in their unprotected forms, leading to the one-step formation of unprotected polysaccharides in aqueous media. Building on the above ideas, we have recently reported the synthesis of amylose-grafted abundant polysaccharides, which have the structure of such the abundant polysaccharide main chains covalently linked to amylose graft chains (Kaneko, Matsuda, & Kadokawa, 2007; Matsuda, Kaneko, & Kadokawa, 2007; Omagari, Matsuda, Kaneko, & Kadokawa, 2009). The synthetic approach

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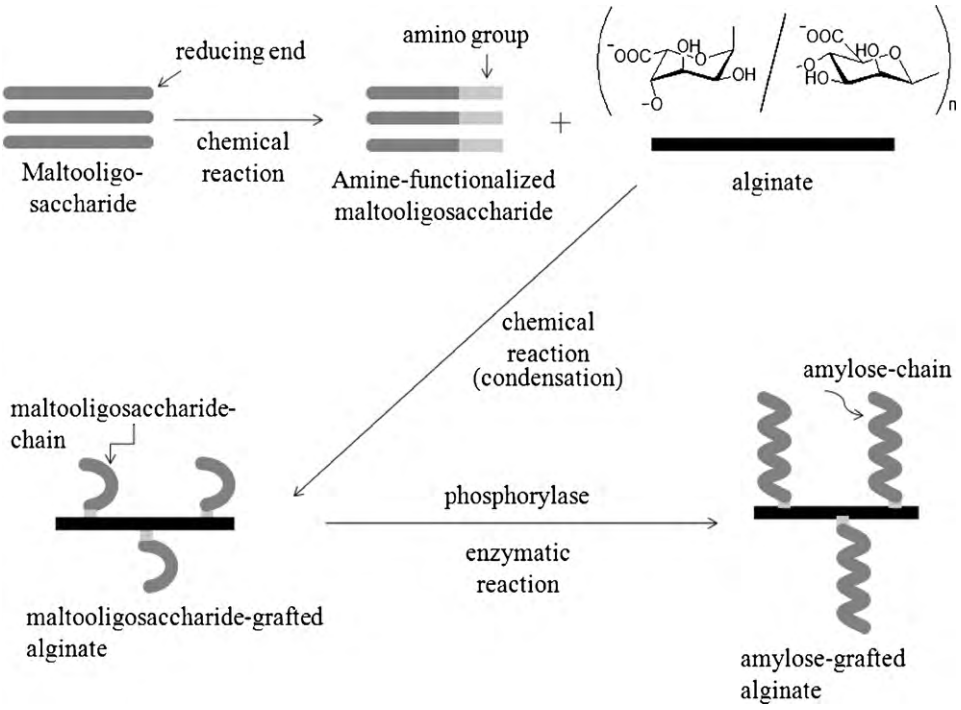


Scheme 1. Chemoenzymatic synthesis of amylose-grafted alginate.

to their materials was performed by a combined method of an enzymatic polymerization forming amylose with the chemical reaction (chemoenzymatic method). Amylose can be prepared by a phosphorylase-catalyzed enzymatic polymerization of glucose 1-phosphate (G-1-P) (Ziegast & Pfannemüller, 1987). This polymerization is initiated from a maltooligosaccharide primer. Then, the propagation proceeds through the following reversible reaction to produce a $(1 \rightarrow 4)$-α-glucan chain, that is amylose; $[(\alpha, 1 \rightarrow 4)\text{-G}]_n + \text{G-1-P} \leftrightarrows [(\alpha, 1 \rightarrow 4)\text{-G}]_{n+1} + \text{P}$. In this reaction, a glucose unit is transferred from G-1-P to the non-reducing 4-OH terminus of a $(1 \rightarrow 4)$-α-glucan chain, resulting in inorganic phosphate (P). Thus, the chemoenzymatic method was achieved by the introduction of maltooligosaccharide primers to the abundant polysaccharide chain (chemical reaction) and the subsequent phosphorylase-catalyzed polymerization of G-1-P (enzymatic reaction) from the primers on the product, leading to the amylose-grafted abundant polysaccharides. For the introduction of the primers to the polysaccharide main-chain, a reductive amination was employed because it had been reported as the reaction which efficiently proceeded between a reducing end of the saccharide chain and aminopolysaccharides (Yalpani & Hall, 1984). For example, we have synthesized the amylose-grafted aminopolysacharides such as chitosan and chitin by means of the above chemoenzymatic approach (Kaneko et al., 2007; Matsuda et al., 2007). The same method was also applied to cellulose main-chain. Because cellulose did not contain amino groups, an amine-functionalized cellulose was first synthesized. Then, the same procedures as above were performed to give the amylose-grafted cellulose (Omagari et al., 2009).

We have continuously been studying on the chemoenzymatic method starting from the other types of polysaccharides besides the polysaccharides having amino groups. For example, we recently attempted to employ the anionic polysaccharides like alginic acid for chemoenzymatic modification resulting in the corresponding amylose-grafting heteropolysaccharides. For this approach, the following reaction steps have been considered (Scheme 1). First, a maltooligosaccharide derivative having an amino group at the reducing end is prepared, which is condensed with the carboxylates of the alginate to give a maltooligosaccharide-grafted alginate. Then, the phosphorylase-catalyzed polymerization of G-1-P from the primer chains is performed to give an amylose-grafted alginate. On the basis of the reaction scheme, in this paper, we report the chemoenzymatic synthesis of the amylose-grafted alginate. Furthermore, formation of the beads from the product by treatment with $CaCl_2$ aqueous solution and enzymatic disintegratability of the beads by amylase-catalyzed hydrolysis of the amylose graft chains were investigated. By means of this property, effect of the enzymatic hydrolysis on the release of an entrapped dye molecule from inside of the beads was also studied. Because of the enzymatic disintegratability by amylase, the present materials can be expected as the candidate of the drug carriers for drug delivery systems in the future.

2. Experimental part

2.1. Materials

Sodium alginate (viscosity; 80–120 cps) was purchased from Wako Pure Chemical Industries, Osaka, Japan. Maltoheptaose lactone was prepared from maltoheptaose according to the literature procedure (Kobayashi, Kamiya, & Enomoto, 1996). 2-Azidoethylamine was prepared by azidation of 2-bromoethylamine hydrobromide using sodium azide according to the literature procedure (Benalil, Carboni, & Vaulitier, 1991). Phosphorylase was supplied from Ezaki Glico Co. Ltd. (Yanase, Takata, Fujii, Takaha, & Kuriki, 2005). β-Amylase from soybean (18,000 unit/g) was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. Other reagents and solvents were used as received.

2.2. Synthesis of amine-functionalized maltooligosaccharide (1)

Under argon, a mixture of maltoheptaose lactone (0.576 g, 0.500 mmol) and 2-azidoethylamine (0.861 g, 10.0 mmol) was heated at 70 °C for 48 h. The resulting solution was poured into a large amount of acetone to precipitate a crude product, which was filtered, washed with acetone and dried under reduced pres-

Table 1
Results of enzymatic polymerization of G-1-P from **2**.

Run	Feed molar ratio G-1-P to **2**	C/N elemental ratio of **3**[a]	Average DP of amylose graft chain on **3**[a]
1	50	134.3	34
2	100	180.4	49
3	200	320.3	96

[a] Determined by CHN elemental analysis. Average DP is the average number of the repeating glucose residues in an amylose chain.

sure. To purify the crude product, it (0.500 g) was once acetylated using acetic anhydride (2.00 mL, 21.2 mmol) in a mixed solvent of DMF and pyridine (3.0 mL, 1:1 (v/v)) at 40 °C for 18 h. The resulting reaction mixture was diluted with chloroform and the solution was successively washed with 1.0 mol/L HCl aqueous solution, saturated $NaHCO_3$ aqueous solution, and water. The chloroform layer was concentrated and the residue was subjected to a column chromatography on silica gel with an eluent of chloroform and methanol (100:1 (v/v)) to give an acetylated product. The material (0.900 g, 0.408 mmol) was dissolved in a mixed solvent of methanol and DMSO (6.0 mL, 1:1 (v/v)) and treated with sodium methoxide (0.513 g, 9.50 mmol) at room temperature for 10 h. The reaction solution was treated with cation-exchange resin (Amberlite IR-120B NA H^+ form) for 10 min and the resin was filtered off. The filtrate was poured into a large amount of acetone to precipitate the product. The precipitate was isolated by filtration, washed with methanol, and dried under reduced pressure to give an azide-functionalized maltooligosaccharide (0.346 g, 0.280 mmol) in 56.0% yield. ^{1}H NMR (D_2O) δ 3.45 (t, CH_2–N_3, J = 2.7 Hz, 2H), 3.41–4.07 (br m, CH_2–NHC═O, –CH(O-D-glu)–CH(OH)–CH_2OH, H2–H6 of D-glu, 42H), 4.19 (dd, C(C═O)CH(OH)CH(OH)–, J = 2.7 and 6.4 Hz, 1H), 4.32 (d, C(C═O)CH(OH)–, J = 2.7 Hz, 1H), 5.17, 5.40 (2d, anomeric protons, J = 4.1 and 3.2 Hz, respectively, 6H); IR 2113 cm^{-1} (–N_3). Under argon, to a solution of the purified material (0.320 g, 0.259 mmol) in DMSO (3.2 mL) was added sodium borohydride (0.245 g, 6.47 mmol) and the mixture was stirred at 60 °C for 24 h. The reaction mixture was poured into a large amount of methanol to precipitate the product. The precipitate was isolated by filtration and dried under reduced pressure to give **1** (0.246 g, 0.203 mmol) in 78.4% yield. ^{1}H NMR (D_2O) δ 3.04 (t, CH_2–NH_2, J = 5.5 Hz, 2H), 3.41–4.07 (br m, CH_2–NHC═O, –CH(O-D-glu)–CH(OH)–CH_2OH, H2–H6 of D-glu, 42H), 4.40 (m, C(C═O)CH(OH)CH(OH)–, 2H), 5.12, 5.39 (2d, anomeric protons, J = 3.4 and 3.4 Hz, respectively, 6H).

2.3. Synthesis of maltooligosaccharide-grafted alginate (2)

To a solution of sodium alginate (0.396 g, 2.00 mmol) in water (10.0 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) (0.0380 g, 0.200 mmol) and *N*-hydroxysuccinimide (NHS) (0.0230 g, 0.200 mmol), and the mixture was stirred at room temperature for 1 h. Then, **1** (0.242 g, 0.200 mmol) was added to the solution and the mixture was stirred at room temperature for 2 h. After the reaction solution was dialyzed in a dialysis bag (molecular weight cut off: 12,000–14,000) against water, the obtained material was purified further by precipitation into methanol. The precipitate was isolated by filtration, washed with DMSO and acetone, and dried under reduced pressure to give **2** (0.456 g). ^{1}H NMR (D_2O) δ 3.30–4.30 (br, sugar protons of H2–H6), 4.33, 5.02 (br, H1 of alginate), 5.36 (br, H1 of maltooligosaccharide).

2.4. Synthesis of amylose-grafted alginate (3)

A typical experimental procedure for the synthesis of **3** was as follows (run 2, Table 1). Maltooligosaccharide-grafted alginate (**2**) (0.0650 g) was dissolved in aqueous acetic acid (0.2 mol/L, 1.0 mL), and then sodium acetate buffer (0.2 mol/L, pH 6.2, 4.0 mL) and sodium salt of G-1-P (0.608 g, 2.00 mmol) were added to this solution. After the pH value of the solution was adjusted to 6.2 by addition of 0.2 mol/L aqueous acetic acid, phosphorylase (potato, EC 2.4.1.1, ca. 42 unit) was added to this solution, and the solution was stirred at 40–45 °C for 7 h. The reaction mixture was dialyzed in a dialysis bag (molecular weight cut off: 12,000–14,000) against 1.0 mol/L NaOH aqueous solution and poured into methanol to precipitate the product. The precipitate was isolated by filtration, washed with methanol, DMSO, and acetone and dried under reduced pressure to give **3** (0.185 g). ^{1}H NMR (1.0 mol/L NaOD/D_2O) δ 3.27–4.29 (br, sugar protons of H2–H6), 4.44, 5.03 (br, H1 of alginate), 5.43 (br, H1 of amylose).

2.5. Preparation of beads from 3 and their enzymatic disintegration

A solution of **3** (run 2, Table 1, 10 mg) in 0.5 mol/L NaOH aqueous solution (0.10 mL) was slowly added to 0.5 mol/L $CaCl_2$ aqueous solution (1.0 mL) to form beads (diameter; ca. 5 mm), which were washed with water. The beads and β-amylase (EC 3.2.1.2, 180 unit) were allowed to stand in a stirred sodium acetate buffer (0.2 mol/L, pH 6.2, 5.0 mL) at 40 °C for 6 h.

2.6. Preparation of fluorescein-entrapping beads from 3 and release experiment

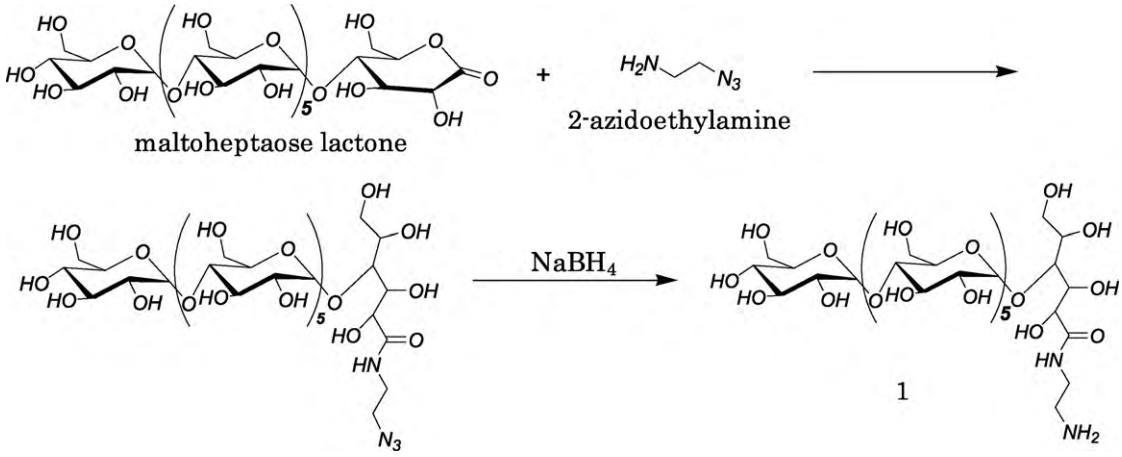
A solution of **3** (run 2, Table 1, 10 mg) and fluorescein (0.10 mg, 0.30 μmol) in 0.5 mol/L NaOH aqueous solution (0.10 mL) was slowly added to 0.5 mol/L $CaCl_2$ aqueous solution (1.0 mL) to form beads, which were washed with water. Amounts of entrapped fluorescein were calculated on the basis of amounts of fluorescein present in $CaCl_2$ aqueous solution to be 5–6 μg. The beads were allowed to stand in the presence or absence of β-amylase (180 unit) in a stirred sodium acetate buffer (0.2 mol/L, pH 6.2, 10.0 mL) at 40 °C. The reaction solution (0.10 mL) was taken at each time and diluted with 0.1 mol/L NaOH aqueous solution to 10 mL. The fluorescence spectrum of the solution was measured and the released amount of fluorescein was calculated by the intensity of an emission maximum at 514 nm by excitation at 484 nm.

2.7. Measurements

^{1}H NMR spectra were recorded on a JEOL ECX-400 spectrometer. IR spectra were recorded on a SHIMADZU FTIR-8400 spectrometer. Elemental analysis was performed using a PerkinElmer 2400 II CHN element analyzer. TGA measurements were performed on a SII TG/DTA 6200 at a heating rate of 10 °C/min. Fluorescence spectra were recorded on a Jasco FP-6300 fluorometer.

3. Results and discussion

For the preparation of the desired amylose-grafted alginate by the chemoenzymatic method as shown in Scheme 1, an amino group has necessarily been introduced to a reducing end of a maltooligosaccharide because it is reacted with the carboxylates of the alginate by condensation, giving rise to the maltooligosaccharide-grafted alginate as a key compound in this study. Therefore, we first performed the synthesis of an amine-functionalized maltooligosaccharide (**1**) by reaction of maltoheptalose lactone with 2-azidoethylamine, followed by reduction by sodium borohydride (Scheme 2). The reaction and work-up procedure were conducted as described in Section 2 and the structure of the isolated product was confirmed by the ^{1}H NMR and IR spectra to be **1**.

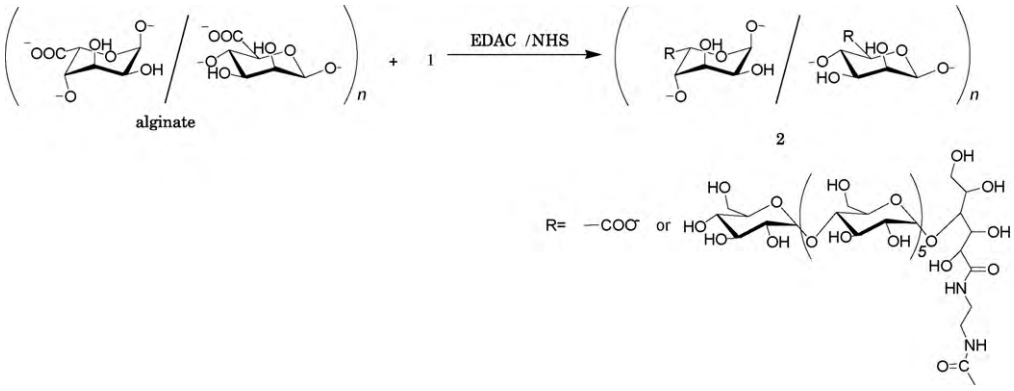


Scheme 2. Preparation of amine-functionalized maltooligosaccharide (**1**).

The condensation of **1** with the carboxylates of sodium alginate was carried out using a condensing agent of EDAC/NHS in water at room temperature to produce a maltooligosaccharide-grafted alginate (**2**) (Scheme 3) (Tada, Tanabe, Tachibana, & Yamauchi, 2007). The structure of the isolated product was characterized by the ^{1}H NMR spectrum and the elemental analysis. The ^{1}H NMR spectrum of the product in D_2O (Fig. 1a) exhibits not only signals at δ 4.33 and 5.02 arising from the H1 of the alginate but also a signal at δ 5.36 that is attributable to the H1 of maltooligosaccharide, indicating that the product has the structure of **2**. The functionality of maltooligosaccharide to the alginate main-chain was calculated by the elemental analysis to be 9.6%.

The synthesis of an amylose-grafted alginate (**3**) was achieved by the phosphorylase-catalyzed enzymatic polymerization of G-1-P from the non-reducing ends of the maltooligosaccharide graft chains on **2** in 0.2 mol/L sodium acetate buffer at 40–45 °C (Scheme 4). The product was isolated by the dialysis against 1.0 mol/L NaOH aqueous solution and purified further by the precipitation into methanol. The isolated product was soluble in the alkaline aqueous solution, and thus, characterized by the ^{1}H NMR spectrum measured in 1.0 mol/L NaOD/D_2O (Fig. 1b, run 2 in Table 1). The integrated ratio of the signal due to H1 of α-glucan (δ 5.43) to the signals due to H1 of alginate (δ 4.44 and 5.03) increases compared with that in the ^{1}H NMR spectrum of **2** (Fig. 1a). The result indicates the progress of the enzymatic chain-elongation reaction from the maltooligosaccharide graft chains. Because alginate, G-1-P, and buffer were used as their sodium salts in this study, it was considered that the produced amylose-grafted alginate mainly had its sodium salt form. The average degree of polymerization (DP) of the amylose graft chains was controlled by changing the feed ratios of G-1-P to **2** for the enzymatic polymerization process. The DPs of amylose graft chains on **3** were calculated on the basis of the elemental analysis data and the functionality of maltooligosaccharide to the alginate main-chain in **2** (9.6%). The C/N elemental ratios of **3**, which were prepared in feed molar ratios of G-1-P to maltooligosaccharide on **2** = 50, 100, and 200, were obtained as 134.3, 180.4, and 320.3, respectively. From these data, the DPs of the amylose graft chains were estimated to be ca. 34, 49, and 96, respectively (Table 1), indicating that the DPs of the amylose graft chains depended on the feed ratios of G-1-P to **2**.

The preparation of beads from **3** was attempted by adding an alkaline solution of **3** into $CaCl_2$ aqueous solution according to the well-known procedure for formation of the general calcium alginate beads (Prasad & Kadokawa, 2009). As shown in Fig. 2a–c, the beads were obtained from the samples of run 1 and 2, whereas the sample of run 3 did not afford formation of beads. This result indicated that the longer amylose graft chains attached to the alginate main-chain disturbed the formation of the stable matrices composed the cross-linked calcium alginates, resulting in difficulty in the formation of beads. Furthermore, the beads obtained from the sample of run 2 were degraded a little when they were soaked in acidic (5.0 mol/L HCl aq.) or alkaline (5.0 mol/L NaOH aq.) aqueous solution. Generally, the calcium alginate beads are stable in large range of pH values. The above observations suggested that the presence of amylose graft chains strongly affected the property of the beads as well as the behavior of their formation. The thermal stability of the present beads was evaluated by the TGA analysis, which was compared with that of the general calcium alginate beads and



Scheme 3. Synthesis of maltooligosaccharide-grafted alginate (**2**).

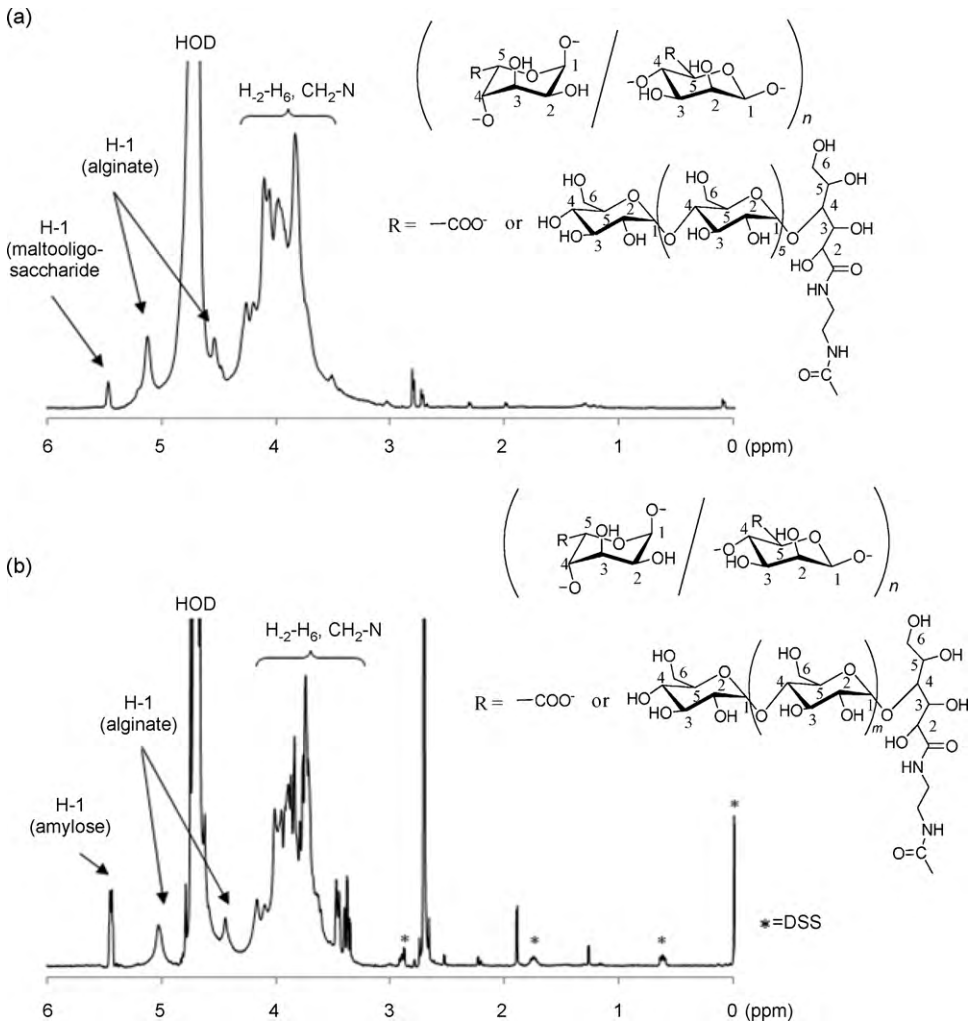
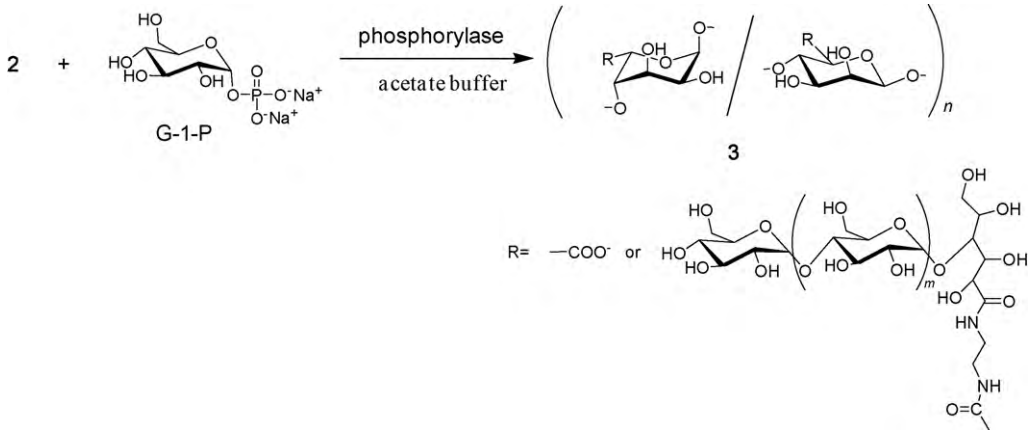


Fig. 1. ^{1}H NMR spectra of **2** in D_2O (a) and **3** in 1.0 mol/L NaOD/D_2O (b).

amylose (Fig. 3). The TGA curve of the present beads from the sample of run 2 exhibited an onset of weight loss at around 210 °C (Fig. 3a), which was between that of the calcium alginate beads (Fig. 3b, ca. 180 °C) and amylose (Fig. 3c, ca. 300 °C). These results suggested enhancement of the thermal stability of the beads by covalently incorporating amylose chains on alginates.

Enzymatic disintegradability of the beads was performed by β-amylase-catalyzed reaction; β-amylase is an enzyme that catalyzes an exo-type hydrolysis at the non-reducing end of α-(1 → 4)-glucans to form a maltose of disaccharide. When the beads from the sample of run 2 was kept standing in the presence of β-amylase in a stirred acetate buffer at 40 °C for 6 h, the solution gradually became turbid (Fig. 2d), indicating disintegration of the beads. On the other hand, the phenomenon was not observed from the same experimental procedure in the absence of β-amylase. The ^{1}HNMR spectrum of the solution fraction obtained by the for-



Scheme 4. Synthesis of amylose-grafted alginate (**3**).

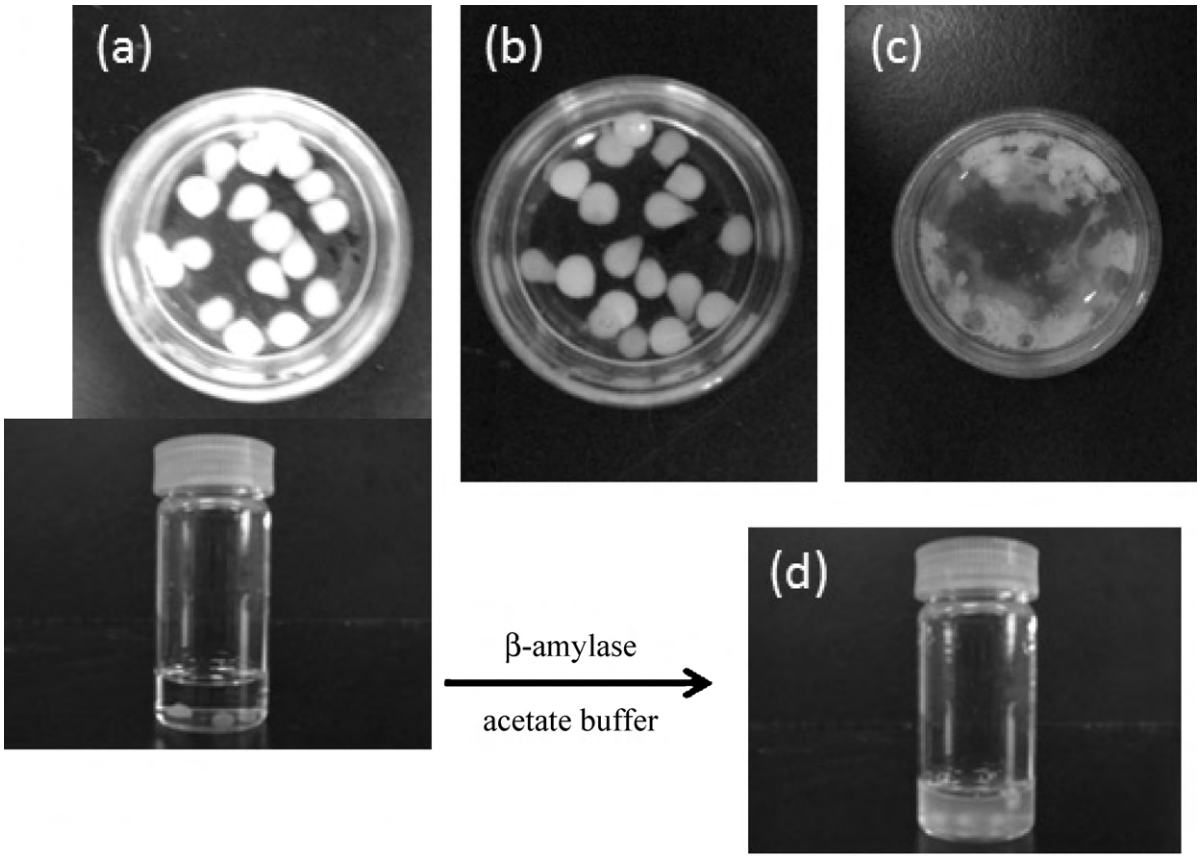


Fig. 2. Photographs of beads from the samples of run 1–3 (a–c) by treatment with $CaCl_2$ aqueous solution and its disintegration by β-amylase (d).

mer experiment showed the signals due to maltose as follows; δ 3.26–4.19 (m, H2–H6, H2′–H6′, 12H), 4.65 and 5.22 (2d, $H1_\beta$, J = 7.8 Hz and $H1_\alpha$, J = 3.7 Hz, respectively, total 1H), 5.40 (d, H1′, J = 3.7 Hz, 1H), whereas no signals due to maltose were detected in the ^{1}HNMR spectrum from the latter experiment. These data suggested that the disintegration of the beads was caused by the enzymatic hydrolysis of the amylose graft chains present in the beads matrices. It was calculated from the amount of the produced maltose that 8.9% of the amylose graft chains were hydrolyzed by the above enzymatic experiment. Then, effect of the enzymatic disintegration of the beads on release of a model substrate from inside of the beads was examined. We selected fluorescein as a suitable model dye entrapped in the beads because its strong fluorescence emission gave us a facile detection by the fluorescence measurement. The beads entrapping fluorescein were prepared from an alkaline solution of **3** (run 2, Table 1) coexisting fluorescein by the same procedure as described above. The amount of fluorescein in the beads was calculated based on the quantification of fluorescein, which was not entrapped. For further analysis, the resulting beads were crushed and subjected to Soxhlet extraction with methanol to extract the entrapped fluorescein. Consequently, most amount of fluorescein was extracted by the above experiment, suggesting that fluorescein was physically bound inside the beads. The obtained beads were allowed to stand in the presence of β-amylase in the stirred acetate buffer solution at 40 °C and the amount of the released fluorescein was estimated by the intensity of an emission maximum at 514 nm

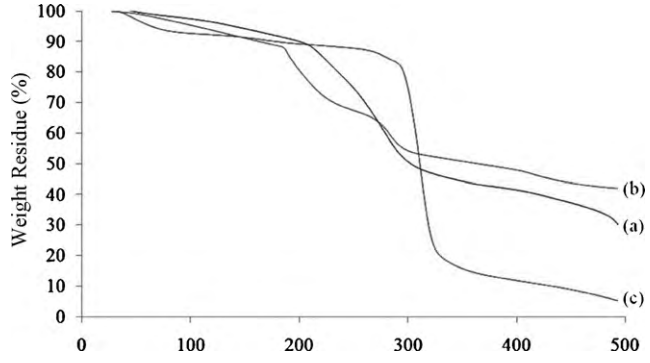


Fig. 3. TGA curves of beads from sample 2 (a), calcium alginate beads (b), and amylose (c).

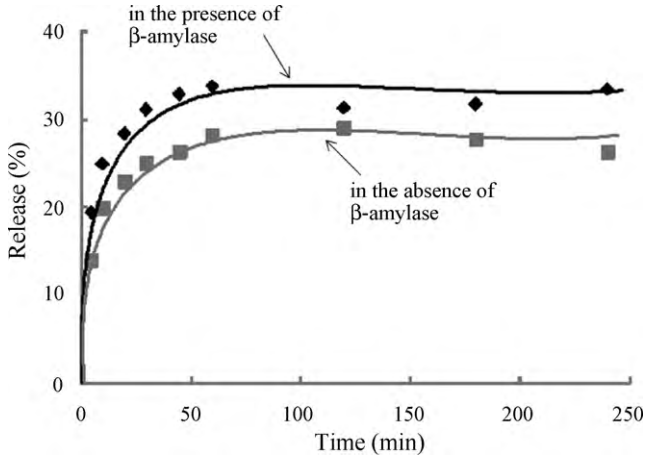


Fig. 4. Release profiles of fluorescein from inside of the beads in the presence and absence of β-amylase.

excited at 484 nm in the fluorescence spectrum of the solution fraction at each time. Fig. 4 shows the releasing profile of fluorescein from the beads by treatment of β-amylase in comparison with a control experiment in the absence of the enzyme. Both graphs rapidly escalated for 0–50 min and were flat at around 100 min. The enhancement of the release rate by the enzymatic treatment was observed, which was reasonably explained by the hydrolysis of the amylose graft chains in the beads by β-amylase.

4. Conclusions

We reported herein the chemoenzymatic synthesis of amylose-grafted alginate. The amine-functionalized maltooligosaccharide (**1**), which was prepared from maltoheptaose lactone, was introduced to sodium alginate by condensation to give the maltooligosaccharide-grafted alginate (**2**). Then, the phosphorylase-catalyzed enzymatic polymerization of G-1-P from the graft chain ends on **2** was performed to obtain the amylose-grafted alginate (**3**). The DPs of the amylose graft chains depended on the feed ratios of G-1-P to **2**. The beads were prepared from **3** by addition of its alkaline solution into $CaCl_2$ aqueous solution. The obtained beads exhibited disintegratable property by β-amylase. Furthermore, the enhancement of the releasing rate of the entrapped dye compound from inside of the beads by the enzyme treatment was observed, which was reasonably explained by the hydrolysis of the amylose graft chains in the beads by β-amylase.

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References

Benalil, A., Carboni, B., & Vaulitier, M. (1991). Synthesis of 1,2-aminoazides. Conversion to unsymmetrical vicinal diamines by catalytic hydrogenation or reductive alkylation with dichloroboranes. *Tetrahedron*, *47*, 8177–8194.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2006). *Biochemistry* (6th international ed.). New York: W.H. Freeman & Co. (Chapter 11).

Kaneko, Y., Matsuda, S., & Kadokawa, J. (2007). Chemoenzymatic syntheses of amylose-grafted chitin and chitosan. *Biomacromolecules*, *8*, 3959–3964.

Kobayashi, K., Kamiya, S., & Enomoto, N. (1996). Amylose-carrying styrene macromonomer and its homo- and copolymers: Synthesis via enzyme-catalyzed polymerization and complex formation with iodine. *Macromolecules*, *29*, 8670–8676.

Kobayashi, S. (2007). New developments of polysaccharide synthesis via enzymatic polymerization. *Proceedings of the Japan Academy Series B-Physical and Biological Sciences*, *83*, 215–247.

Kobayashi, S., Ohmae, M., Fujikawa, S., & Ochiai, H. (2005). Enzymatic precision polymerization for synthesis of glycosaminoglycans and their derivatives. *Macromolecular Symposia*, *226*, 147–156.

Kobayashi, S., Uyama, H., & Kimura, S. (2001). Enzymatic polymerization. *Chemical Reviews*, *101*, 3793–3818.

Matsuda, S., Kaneko, Y., & Kadokawa, J. (2007). Chemoenzymatic synthesis of amylose-grafted chitosan. *Macromolecular Rapid Communications*, *28*, 863–867.

Omagari, Y., Matsuda, S., Kaneko, Y., & Kadokawa, J. (2009). Chemoenzymatic synthesis of amylose-grafted cellulose. *Macromolecular Bioscience*, *9*, 450–455.

Prasad, K., & Kadokawa, J. (2009). Alginate-based blends and nano/microbeads. In B. H. A. Rehm (Ed.), *Alginates: Biology and applications* (pp. 175–210). Berlin: Springer.

Schuerch, C. (1986). Polysaccharides. In H. F. Mark, N. Bilkales, & C. G. Overberger (Eds.), *Encyclopedia of polymer science and engineering* (2nd ed., Vol. 13, pp. 87–162). New York: John Wiley & Sons.

Shoda, S., Izumi, R., & Fujita, M. (2003). Green process in glycotechnology. *Bulletin of the Chemical Society of Japan*, *76*, 1–13.

Tada, D., Tanabe, T., Tachibana, A., & Yamauchi, K. (2007). Albumin-crosslinked alginate hydrogels as sustained drug release carrier. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, *C27*, 870–874.

Yalpani, M., & Hall, L. D. (1984). Some chemical and analytical aspects of polysaccharide modifications. III. Formation of branched-chain, soluble chitosan derivatives. *Macromolecules*, *17*, 272–281.

Yanase, M., Takata, H., Fujii, K., Takaha, T., & Kuriki, T. (2005). Cumulative effect of amino acid replacements results in enhanced thermostability of potato type L α-glucan phosphorylase. *Applied Environmental Microbiology*, *71*, 5433–5439.

Ziegast, G., & Pfannemüller, B. (1987). Phosphorolytic syntheses with di-, oligo- and multi-functional primers. *Carbohydrate Research*, *160*, 185–204.