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Preparation of Amylose-Carboxymethyl Cellulose Conjugated Supramolecular Networks by Phosphorylase-Catalyzed Enzymatic Polymerization

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Abstract: Enzymatic polymerization has been noted as a powerful method to precisely synthesize polymers with complicated structures, such as polysaccharides, which are not commonly prepared by conventional polymerization. Phosphorylase is one of the enzymes which have been used to practically synthesize well-defined polysaccharides. The phosphorylase-catalyzed enzymatic polymerization is conducted using α-D-glucose 1-phosphate as a monomer, and maltooligosaccharide as a primer, respectively, to obtain amylose. Amylose is known to form supramolecules owing to its helical conformation, that is, inclusion complex and double helix, in which the formation is depended on whether a guest molecule is present or not. In this paper, we would like to report the preparation of amylose-carboxymethyl cellulose (CMC) conjugated supramolecular networks, by the phosphorylase-catalyzed enzymatic polymerization, using maltoheptaose primer-grafted CMC. When the enzymatic polymerization was carried out using the graft copolymer, either in the presence or in the absence of a guest polymer poly (ε-caprolactone) (PCL), the enzymatically elongated amylose chains from the primers on the CMC main-chain formed double helixes or inclusion complexes, depending on the amounts of PCL, which acted as cross-linking points for the construction of network structures. Accordingly, the reaction mixtures totally turned into hydrogels, regardless of the structures of supramolecular cross-linking points.

Keywords: amylose; carboxymethyl cellulose; enzymatic polymerization; phosphorylase; supramolecule

1. Introduction

Enzyme-catalyzed reactions have been employed to precisely synthesize biological and bio-related polymeric molecules with regular structures, which are identified as a term of enzymatic polymerization because they strictly progress with controlled regio-, stereo-, and conformational arrangements [1–4]. Polysaccharides are the representative molecules, which have been beneficially constructed according to such advantages of enzymatic catalysis [5–7]. They are comprising very complicated structures which are comprised of a variety of monosaccharide units, linked through specific types of glycosidic linkages, with highly controlled regio- and stereoarrangements. For example, amylose with a well-defined structure is comprised of α(1→4)-linked glucose (G) repeating units, and synthesized by the phosphorylase-catalyzed enzymatic polymerization of the α-D-glucose 1-phosphate (G-1-P) monomer [6–10]. The polymerization is precisely initiated at the non-reducing end of a maltooligosaccharide primer, and G residues are consecutively transferred from the monomers to the non-reducing propagating chain end. Therefore, the molecular weights
of the produced amyloses can be controlled by G-1-P (monomer)/maltooligosaccharide (primer) feed ratios. Amylose is an energy resource and one of the components of starch. It is identified as a functional polymeric material, owing to its left-handed helical conformation which readily forms controlled double helical assembly in water [11,12]. Furthermore, amylose acts as a host molecule and includes hydrophobic guest molecules with relatively small sizes, which reside within a cavity of the helix as its hydrophobic nature, forms inclusion complexes [13]. We have found that when the phosphorylase-catalyzed enzymatic polymerization is conducted in the presence of guest polymers with suitable hydrophobicity such as poly(ε-caprolactone) (PCL, a hydrophobic polyester), and dispersed in aqueous polymerization solvents which have larger sizes than those of the common guest molecules, the enzymatically propagating amylose chain helically interacts with the polymers to produce amylose-polymer inclusion complexes [14,15]. The elongation from the short maltooligosaccharide primer to the longer amylose chain, through polymerization, is conceived to provide a sufficient dynamic field for efficient complexation with the guest polymers. This system’s propagation likely mirrors the way that the vines of plants grow with twining around a rod. Accordingly, we proposed that this polymerization method, for the construction of amylose-polymer inclusion complexes, is called “vine-twining polymerization” [16–22].

As the reducing end of the maltooligosaccharide primer (an opposite chain end to the propagation) does not participate in the enzymatic reaction, the phosphorylase-catalyzed enzymatic polymerization can be carried out using polymeric primers. During this process, the reducing ends were covalently immobilized on polymeric chains to produce amylose-grafted polymeric materials [4–7,18,21,23–25]. For example, a maltoheptaose (G7)-grafted poly(γ-glutamic acid) (PGA) has been used as the polymeric primer for enzymatic polymerization; PGA is a well-known natural polypeptide with moisturizing and high water retention properties [26,27] which has been considered to contribute to the formation of functional materials. Indeed, the enzymatically elongated amylose chains formed double helical assemblies among the PGA main-chains to construct a supramolecular network structure which resulted in hydrogelation of the polymerization mixture [28]. Furthermore, an attempt was also made to fabricate supramolecular networks comprising amylose-PCL inclusion complexes as cross-linking points by the vine-twining polymerization in the presence of PCL, using G7-grafted PGA [29,30]. With increasing PCL/G7 feed ratios, the reaction mixtures hardly turned into the hydrogels and aggregates were in turn obtained in the polymerization mixtures. This is because the smaller networks, mostly composed of the amylose-PCL inclusion complexes, were formed according to the vine-twining polymerization manner in the presence of higher feed ratios of PCL, which did not have the potential to stably include sufficient volumes of water for hydrogelation.

In this study, we employed carboxymethyl cellulose (CMC) as the main-chain polymer for grafting the G7 primers, in place of PGA, to produce supramolecular network hydrogels as the vine-twining polymerization products with PCL (Figure 1a). CMC is practically used as a viscosity modifier and thickener in food industries, which have been speculated by us to be appropriate as a component in supramolecular materials [31]. We found, in the present study, that regardless of the PCL/G7 feed ratios, the enzymatic polymerization mixtures totally turned into the hydrogels. In the previous publications related to the present study, we have briefly reported phosphorylase-catalyzed enzymatic polymerization using G7-grafted CMC, which forms a hydrogel with cross-linking points from amylosic double helixes in the polymerization mixture [32,33]. The produced amylose-grafted CMC, additionally, formed films with the hierarchically constructed nanostructure.
Figure 1. (a) Image for preparation of supramolecular network materials by means of amylose helical assemblies formed in phosphorylase-catalyzed enzymatic polymerization, and (b) photographs of reaction mixtures.

2. Results and Discussion

Prior to demonstrating the phosphorylase-catalyzed enzymatic polymerization, G7-grafted CMC was prepared by condensation of carboxyates in CMC (molecular weight (MW) = $2.5 \times 10^6$), degree of carboxymethylation (DC) = 0.7, with an amine-functionalized G7 in the presence of the condensing agent, comprising 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) in NaOH aq. at room temperature according to our previous literature procedure [32]. The structure of the product, which was isolated by dialysis against water and a subsequent precipitation procedure into methanol, was confirmed by the $^1$H NMR measurement in D$_2$O. The degree of substitution (DS) value of the G7 primers, with total repeating units of CMC, was estimated by the integrated ratio of signals assignable to anomeric protons in the G7 graft-chains to a signal ascribable to anomeric protons in the CMC main-chain to be 3.8%.

The phosphorylase-catalyzed enzymatic polymerization of G-1-P, using the resulting G7-grafted CMC, was then carried out in different PCL/G7 feed ratios (0, 3, 5, and 10) in aqueous acetate buffer (Figure 1a). The consistent G7 concentration (= 3.0 µmol/0.4 mL acetate buffer) and G-1-P/G7 feed ratio (=100) were employed in all runs. Regardless of the PCL/G7 feed ratios, the reaction mixtures totally turned into the hydrogels after maintaining for 24 h at 45 °C (Figure 1b). On the other hand, the phosphorylase-catalyzed enzymatic polymerization, using G7-grafted PGA with similar MW and DS values (1.5 − 2.5 $\times 10^6$ and 3.0%, respectively), under the same conditions of run 3, did not result in hydrogelation. These results strongly suggested that CMC was a better component than PGA for conjugation with amylose to construct the amylosic supramolecular network.

The resulting hydrogels were taken out of the vessels, washed with water and acetone several times for purification (the removal of un-included PCL, phosphorylase, unreacted G-1-P, and buffer salt), and lyophilized. The monomer conversions, amylose molecular weights ($M_n$), amylose contents, and water contents, were estimated by weights of the products (Table 1). By increasing the PCL/G7 feed ratios, the monomer conversions, molecular weights, and water contents decreased at the same
tendency as had appeared in the previous study using G\textsubscript{7}-grafted PGA [29]. As already discussed in the previous report [29], inclusion complexes were predominantly formed in the presence of larger amounts of PCL, which resulted in the prevention of further progress of the enzymatic polymerization to produce the lower molecular weight amylose. Accordingly, the smaller networks were constructed from inclusion complexes with the shorter amylose graft chains as cross-linking points than those obtained from double helixes, resulting in lower water contents of the hydrogels.

Table 1. Preparation of supramolecular network materials by phosphorylase-catalyzed enzymatic polymerization.

| Run | PCL/G\textsubscript{7} | Conversion \textsuperscript{b} (%) | \(M_n\) of Amylose \textsuperscript{b} | Amylose Content \textsuperscript{b} (wt\%) | Water Content \textsuperscript{c} (wt\%) | PCL Content \textsuperscript{d} (wt\%) | IC: DH in Amylose \textsuperscript{e} (wt ratio) |
|-----|----------------------|---------------------|-----------------|-------------------------------|-----------------|-----------------|------------------|
| 1   | 0                    | 93.3                | 16,000          | 70.1                          | 93.2            | 0               | 0:100            |
| 2   | 3                    | 86.7                | 15,000          | 66.6                          | 86.4            | 3.3             | 43.7:56.3        |
| 3   | 5                    | 83.3                | 14,000          | 63.6                          | 85.4            | 5.7             | 80.0:20.0        |
| 4   | 10                   | 80.0                | 14,000          | 61.7                          | 85.2            | 6.8             | 98.4:1.6         |

\textsuperscript{a} Reaction was carried out in aqueous sodium acetate buffer solution (0.4 mL) at 45 °C for 24 h, G\textsubscript{7} = 3.0 µm, G-1-P/G\textsubscript{7} feed ratio = 100. \textsuperscript{b} Determined by weight of product. \textsuperscript{c} Calculated by the weight difference of lyophilized sample from hydrogel. \textsuperscript{d} Determined by weight of recovered poly (\(\varepsilon\)-caprolactone) (PCL). \textsuperscript{e} Determined by PCL content and theoretical PCL/amylose unit ratio in inclusion complex; IC = inclusion complex, DH = double helix.

The formation of either the double helix or inclusion complex, as the amylose assembling structure in the products, was evaluated by the powder X-ray diffraction (XRD) measurement of the lyophilized samples (Figure 2). Because of the absence of PCL, the XRD profile of the sample of run 1 shows the diffraction peaks at around 17° and 22°, ascribed to the double helix (Figure 2a,e). When the PCL/G\textsubscript{7} feed ratio of 3 was employed, the diffraction peaks at 13° and 20°, ascribable to the inclusion complex which are mildly detectable, in addition to those due to the double helix (Figure 2b,f). With increasing the PCL/G\textsubscript{7} feed ratios (=5 and 10), the XRD profiles mostly observed the diffraction peaks assignable to the inclusion complex (Figure 2c,d). The weight ratios of the inclusion complex to the double helix in the lyophilized samples were precisely estimated from weights of the recovered (un-included) PCL present in the acetone fractions. Based on a theoretical PCL/amylose unit ratio (0.16) in the inclusion complex with \(\delta_1\) helix [14], the weight ratios of two amylosic assemblies (inclusion complex and double helix) could be calculated, which increased with increasing the PCL/G\textsubscript{7} feed ratios. These data are in good agreement with the abovementioned XRD results.

The amylosic assembling structures in the supramolecular networks, depending on reaction times, were then evaluated. The phosphorylase-catalyzed enzymatic polymerization, using G\textsubscript{7}-grafted CMC, was conducted under the conditions of run 2 with different reaction times of 0.5, 1, 3, and 5 h. The \(M_n\) values of the amylose graft chains, which were evaluated by weights of the lyophilized samples, increased in accordance with prolonged reaction times of 8000, 11,000, 16,000, and 17,000. The XRD profile of the lyophilized product with shorter reaction time (0.5 h) sorely observed the diffraction peaks ascribable to the inclusion complex (Figure 3a). With prolonged reaction times, the diffraction peaks caused by the double helix gradually appear (Figure 3b–d). These results indicated that the short amylose graft chains included PCL in the cavities at an early stage of the enzymatic polymerization, according to the vine-twining polymerization manner (Figure 4a). At following stages, the formation of the double helices gradually occurred from the longer amylose chains (Figure 4b). The products obtained with different reaction times were further characterized by dynamic viscoelastic measurement. The frequency dependences of storage and loss moduli (\(G'\) and \(G''\), respectively) of all the products before lyophilization observe signature of the typical viscoelastic material with the predominance of the \(G'\) values on the whole frequency range (Figure 4). Furthermore, the profiles in Figure 4 have been similar to those of the hydrogel previously obtained by the phosphorylase-catalyzed enzymatic polymerization using G\textsubscript{7}-grafted PGA under similar conditions with a reaction time of 48 h [29].
This suggests, in enzymatic polymerization using G$_7$-grafted CMC, even with shorter reaction times (0.5–5 h), the hydrogels with comparable property to that using G$_7$-grafted PGA with longer reaction time (48 h), are produced. These results also indicated that CMC was better than PGA as the component for conjugation with amylose to construct the amylosic supramolecular network.

Figure 2. X-ray diffraction (XRD) profiles of (a–d) products by lyophilization of runs 1–4, (e) enzymatically synthesized amylose, and (f) amylose-PCL inclusion complex.

Figure 3. XRD profiles of products by lyophilization of run 2 with reaction times of (a–d) 0.5, 1, 3, and 5.
The macroscopic morphologies of the lyophilized samples of runs 1–4 were then evaluated by the SEM measurement (Figure 5). With increasing the PCL/G7 feed ratios, smaller network morphologies are observed in the SEM images. In the presence of larger amounts of PCL, the inclusion complexes were mostly formed at the early stage, according to the vine-twining polymerization manner, which prevented further progress of the enzymatic polymerization to obtain the smaller networks with short amylose chains upon molecular level, resulting in the hierarchical fabrication of the smaller macroscopic networks (Figure 6a). Meanwhile, in the absence of PCL or the presence of smaller amounts of PCL longer amylose chains were smoothly produced, without prevention by inclusion complexes in the enzymatic polymerization, to form the larger networks at a molecular level (Figure 6b). Therefore, the larger macroscopic networks were hierarchically constructed under these conditions.
3. Materials and Methods

3.1. Materials

A sodium salt form of CMC (MW = 2.5 × 10^6, DC = 0.7) was commercially available from Sigma–Aldrich Chemical Co. Ezaki Glico Co. Ltd., Osaka, Japan kindly supplied thermostable phosphorylase from *Aquifex aeolicus* V5F [10,34,35]. G7-grafted CMC was prepared according to the literature procedure [32]. 1H NMR (D2O) δ 5.15, 5.33 (br s, H1 of G7), 4.41-4.70 (br s, H1 of CMC), 3.10–4.05 (br, sugar protons of H2-H6). The DS for the grafting was determined by the integrated ratio of the H1 signal of G7 to the H1 signal of CMC to be 3.8%. PCL was prepared by ring-opening polymerization of ε-caprolactone initiated with 6-hydroxyhexanoic acid in the presence of scandium trifluoromethanesulfonate catalyst in toluene according to the literature procedure [36]. 1H NMR (CDCl3) δ 4.05-4.12 (br, O=C-C-C-C-CH2-O), 3.62-3.68 (br, CH2-OH), 2.30-2.36 (br, CH2-(C=O)-O), 1.61-1.67 (br, CH2-C-CH2-C=O), 1.38–1.43 (br, CH2-C-C-C=O). The Mn value was estimated by the integrated ratio of the main-chain signals to the terminal signals to be 700. Other reagents and solvents were available commercially and used without further purification.

3.2. Phosphorylase-Catalyzed Enzymatic Polymerization of G-1-P Using G7-Grafted PGA in the Presence of PCL

A typical experimental procedure was as follows (run 4, PCL/G7 = 10). G7-grafted CMC (0.019 g, 0.079 unit mmol, G7; 3.0 µmmol) was first dissolved in an aqueous sodium acetate buffer solution (0.2 mol/L, pH 6.2, 0.4 mL). After G-1-P (disodium salt, 0.91 g, 0.3 mmol, 100 equiv. with primer) and a solution of PCL (7.0 mg, 10.0 µmmol) in acetone (0.06 mL) was mixed to the solution, the mixture was then maintained in the presence of thermostable phosphorylase (24.4 units) for 24 h at 45 °C. For purification, the obtained hydrogel was soaked and washed with water and acetone, in turn, several times (418 mg). The purified material was lyophilized to obtain the product (61.9 mg). The un-included PCL was recovered (16.8 mg) by evaporating the acetone fraction.

3.3. Measurements

The 1H NMR spectra were measured on JEOL ECA600 and ECX400 spectrometers (JEOL, Akishima, Tokyo, Japan). The XRD measurements were conducted on a PANalytical X’Pert Pro MPD diffractometer (PANalytical B.V., EA Almelo, The Netherlands) with Ni-filtered Cu Kα radiation (λ = 0.15418 nm). The dynamic viscoelastic measurements were performed using a rheometer, Rhesosol-G1000 (UBM, Kyoto, Japan). The SEM images were measured using a Hitachi S-4100H electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

4. Conclusions

In this study, we have found that the phosphorylase-catalyzed enzymatic polymerization of G-1-P using G7-grafted CMC is a useful method to produce amylosic supramolecular networks conjugated with other polymeric components, which efficiently form hydrogels. The formation of double helix cross-linking points from the amylose graft chains in the absence of the guest polymer, PCL, gave rise to the construction of the larger network. By increasing the amounts of PCL, inclusion complex cross-linking points were predominantly formed according to the vine-twining polymerization manner, resulting in the formation of the smaller networks. The present supramolecular materials, which are consisting of bio-based and biodegradable polymers, that is, amylose, CMC, and PCL, have the potential to be practically employed as new soft materials in tissue engineering and biomedical applications in the future.

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References

1. Kobayashi, S.; Uyama, H.; Kimura, S. Enzymatic polymerization. Chem. Rev. 2001, 101, 3793–3818. [CrossRef] [PubMed]
2. Kobayashi, S.; Makino, A. Enzymatic polymer synthesis: An opportunity for green polymer chemistry. Chem. Rev. 2009, 109, 5288–5353. [CrossRef] [PubMed]
3. Kadokawa, J.; Kobayashi, S. Polymer synthesis by enzymatic catalysis. Curr. Opin. Chem. Biol. 2010, 14, 145–153. [CrossRef] [PubMed]
4. Shoda, S.; Uyama, H.; Kadokawa, J.; Kimura, S.; Kobayashi, S. Enzymes as green catalysts for precision macromolecular synthesis. Chem. Rev. 2016, 116, 2307–2413. [CrossRef] [PubMed]
5. Kadokawa, J. Precision polysaccharide synthesis catalyzed by enzymes. Chem. Rev. 2011, 111, 4308–4345. [CrossRef] [PubMed]
6. Kadokawa, J. Precision synthesis of functional polysaccharide materials by phosphorylase-catalyzed enzymatic reactions. Polymers 2016, 8, 138. [CrossRef]
7. Kadokawa, J. α-Glucan phosphorylase: A useful catalyst for precision enzymatic synthesis of oligo- and polysaccharides. Curr. Org. Chem. 2017, 21, 1192–1204. [CrossRef]
8. Ziegast, G.; Pfannemuller, B. Linear and star-shaped hybrid polymers. 4. Phosphorolytic syntheses with di-functional, oligo-functional and multifunctional primers. Carbohydr. Res. 1987, 160, 185–204. [CrossRef]
9. Ohdan, K.; Fujii, K.; Yanase, M.; Takaha, T.; Kuriki, T. Enzymatic synthesis of amylose. Biocatal. Biotransform. 2006, 24, 77–81. [CrossRef]
10. Yanase, M.; Takaha, T.; Kuriki, T. α-Glucan phosphorylase and its use in carbohydrate engineering. J. Sci. Food Agric. 2006, 24, 1631–1635. [CrossRef]
11. Imberty, A.; Chanzy, H.; Perez, S.; Buleon, A.; Tran, V. The double-helical nature of the crystalline part of A-starch. J. Mol. Biol. 1988, 201, 365–378. [CrossRef]
12. Imberty, A.; Perez, S. A revisit to the three-dimensional structure of B-type starch. Biopolymers 1988, 27, 1205–1221. [CrossRef]
13. Putseys, J.A.; Lamberts, L.; Delcour, J.A. Amylose-inclusion complexes: Formation, identity and physico-chemical properties. J. Cereal Sci. 2010, 51, 238–247. [CrossRef]
14. Kadokawa, J.; Kaneko, Y.; Nakaya, A.; Tagaya, H. Formation of an amylose-polyester inclusion complex by means of phosphorylase-catalyzed enzymatic polymerization of α-D-glucose 1-phosphate monomer in the presence of poly(epsilon-caprolactone). Macromolecules 2001, 34, 6536–6538. [CrossRef]
15. Kadokawa, J.; Nakaya, A.; Kaneko, Y.; Tagaya, H. Preparation of inclusion complexes between amylose and ester-containing polymers by means of vine-twinning polymerization. Macromol. Chem. Phys. 2003, 204, 1451–1457. [CrossRef]
16. Kaneko, Y.; Kadokawa, J. Vine-twinning polymerization: A new preparation method for well-defined supramolecules composed of amylose and synthetic polymers. Chem. Rec. 2005, 5, 36–46. [CrossRef] [PubMed]
17. Kaneko, Y.; Kadokawa, J. Synthesis of nanostructured bio-related materials by hybridization of synthetic polymers with polysaccharides or saccharide residues. J. Biomater. Sci. Polym. Ed. 2006, 17, 1269–1284. [CrossRef] [PubMed]
18. Kaneko, Y.; Kadokawa, J. Preparation of polymers with well-defined nanostructure in the polymerization field. In Modern Trends in Macromolecular Chemistry; Lee, J.N., Ed.; Nova Science Publishers, Inc.: Hauppauge, NY, USA, 2009; pp. 199–217.
19. Kadokawa, J. Preparation and applications of amylose supramolecules by means of phosphorylase-catalyzed enzymatic polymerization. Polymers 2012, 4, 116–133. [CrossRef]
20. Kadokawa, J. Architecture of amylose supramolecules in form of inclusion complexes by phosphorylase-catalyzed enzymatic polymerization. Biomolecules 2013, 3, 369–385. [CrossRef] [PubMed]
21. Kadokawa, J. Chemoenzymatic synthesis of functional amylosic materials. Pure Appl. Chem. 2014, 86, 701–709. [CrossRef]
22. Orio, S.; Yamamoto, K.; Kadokawa, J. Preparation and material application of amylose-polymer inclusion complexes by enzymatic polymerization approach. Polymers 2017, 9, 729. [CrossRef]
23. Kadokawa, J. Synthesis of amylose-grafted polysaccharide materials by phosphorylase-catalyzed enzymatic polymerization. In Biobased Monomers, Polymers, and Materials; Smith, P.B., Gross, R.A., Eds.; ACS Symposium Series 1105; American Chemical Society: Washington, DC, USA, 2012; pp. 237–255.
24. Kadokawa, J. Synthesis of new polysaccharide materials by phosphorylase-catalyzed enzymatic α-glycosylations using polymeric glycosyl acceptors. In Green Polymer Chemistry: Biocatalysis and Materials II; Cheng, H.N., Gross, R.A., Smith, P.B., Eds.; ACS Symposium Series 1144; American Chemical Society: Washington, DC, USA, 2013; pp. 141–161.
25. Nishimura, T.; Akiyoshi, K. Amylose engineering: Phosphorylase-catalyzed polymerization of functional saccharide primers for glycobiomaterials. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2017, 9. [CrossRef] [PubMed]
26. Bajaj, I.; Singhal, R. Poly (glutamic acid)—An emerging biopolymer of commercial interest. Bioresour. Technol. 2011, 102, 5551–5561. [CrossRef] [PubMed]
27. Ogunleye, A.; Bhat, A.; Irorere, V.U.; Hill, D.; Williams, C.; Radecka, I. Poly-g-glutamic acid: Production, properties and applications. Microbiology 2015, 161, 1–17. [CrossRef] [PubMed]
28. Shouji, T.; Yamamoto, K.; Kadokawa, J. Chemoenzymatic synthesis and self-assembling gelation behavior of amylose-grafted poly(g-glutamic acid). Int. J. Biol. Macromol. 2017, 97, 99–105. [CrossRef] [PubMed]
29. Kadokawa, J.; Shoji, T.; Yamamoto, K. Preparation of supramolecular network materials by means of amylose helical assemblies. Polymer 2018, 140, 73–79. [CrossRef]
30. Orio, S.; Shoji, T.; Yamamoto, K.; Kadokawa, J.-I. Difference in macroscopic morphologies of amylosic supramolecular networks depending on guest polymers in vine-twining polymerization. Polymers 2018, 10, 1277. [CrossRef]
31. Stephen, A.M.; Phillips, G.O.; Williams, P.A. Food Polysaccharides and Their Applications, 2nd ed.; CRC/Taylor & Francis: Boca Raton, FL, USA, 2006; 733p.
32. Kadokawa, J.; Arimura, T.; Takemoto, Y.; Yamamoto, K. Self-assembly of amylose-grafted carboxymethyl cellulose. Carbohydr. Polym. 2012, 90, 1371–1377. [CrossRef] [PubMed]
33. Hatanaka, D.; Takemoto, Y.; Yamamoto, K.; Kadokawa, J. Hierarchically self-assembled nanofiber films from amylose-grafted carboxymethyl cellulose. Fibers 2013, 2, 34–44. [CrossRef]
34. Bhuiyan, S.H.; Rus’d, A.A.; Kitaoa, M.; Hayashi, K. Characterization of a hyperthermostable glycogen phosphorylase from Aquifex aeolicus expressed in Escherichia coli. J. Mol. Catal. B Enzym. 2003, 22, 173–180. [CrossRef]
35. Yanase, M.; Takata, H.; Fujii, K.; Takaha, T.; Kuriki, T. Cumulative effect of amino acid replacements results in enhanced thermostability of potato type L α-glucan phosphorylase. Appl. Environ. Microbiol. 2005, 71, 5433–5439. [CrossRef] [PubMed]
36. Nomura, N.; Taira, A.; Tomioka, T.; Okada, M. A catalytic approach for cationic living polymerization: Sc(OTf)3-catalyzed ring-opening polymerization of lactones. Macromolecules 2000, 33, 1497–1499. [CrossRef]