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Preparation of polysaccharide supramolecular films by vine-twining polymerization approach

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ABSTRACT

In this study, we investigated the preparation of polysaccharide supramolecular films through the formation of inclusion complexes by amylose in vine-twining polymerization using carboxymethyl cellulose-*graft*-poly(ε-caprolactone) (CMC-*g*-PCL) as a new guest polymer. First, hydrogels were prepared by phosphorylase-catalyzed enzymatic polymerization in the presence of CMC-*g*-PCL according to the vine-twining polymerization manner. The XRD result of a powdered sample obtained by lyophilization of the resulting hydrogel indicated the presence of inclusion complexes of amylose with the PCL graft-chains between intermolecular (CMC-*g*-PCL)s, which acted as supramolecular cross-linking points for the hydrogelation. Then, the supramolecular films were obtained by adding water to the powdered samples, followed by drying. The mechanical properties of the selected films examined by tensile testing were superior to those of a CMC film. The effect of the supramolecular cross-linking structures on the mechanical properties of the films was evaluated further by several investigations.

1. Introduction

Natural polysaccharides such as starch and cellulose are the representative abundant biological macromolecules on the earth (Berg, Tymoczko, & Stryer, 2006; Schuerch, 1986) and have widely been studied for their potential to become environmentally benign substitutes for petroleum-based materials because of their eco-friendly and biodegradable properties (Mohanty, Misra, & Drzal, 2002; Rouilly & Rigal, 2002). Amylose is a component of starch and a linear polysaccharide composed of glucose residues linked through α(1→4)-glycosidic bonds (Lenz, 1993). Because amylose can precisely be synthesized by enzymatic polymerization catalyzed by phosphorylase (Fujii et al., 2003; Kitaoka & Hayashi, 2002; Kobayashi & Makino, 2009; Ohdan, Fujii, Yanase, Takaha, & Kuriki, 2006; Seibel, Jördening, & Buchholz, 2006; Yanase, Takaha, & Kuriki, 2006; Ziegast & Pfannemüller, 1987), various amylose-based polysaccharide materials have been prepared by the designed enzymatic approaches by phosphorylase catalysis (Kadokawa & Kaneko, 2013). The phosphorylase-catalyzed enzymatic polymerization is conducted using α-D-glucose 1-phosphate (G-1-P) as a monomer and can be initiated from a non-reducing end of maltooligosaccharide primers such as maltoheptaose (G_7). Then, the propagation proceeds through the following reversible reaction to produce amylose; $(\alpha(1\rightarrow4)\text{-G})_n + \text{G-1-P} \leftrightarrows (\alpha(1\rightarrow4)\text{-G})_{n+1} + \text{P}$. In the reaction, glucose units are successively transferred from G-1-P to a non-reducing 4-OH terminus of a α(1→4)-glucan chain, resulting in the chain-elongation accompanied with the production of inorganic phosphate (P).

Besides the role of amylose in nature is the energy resource as a component of starch, it is also well-known as a functional host molecule because of its helical conformation that constructs hydrophobic field in inside of the cavity (Sarko & Zugenmaier, 1980). Therefore, it allows amylose to form supramolecules with various hydrophobic guest compounds, that is, inclusion complexes, so-called amylose V complexes, by their hydrophobic interactions with the cavity of amylose (Choi & Kim, 1998; Kim, Choi, Zhang, He, & Shih, 1996; Kim, Je, & Melinger, 2006; Sanji, Kato, Kato, & Tanaka, 2005; Sanji, Kato, & Tanaka, 2006a,b). However, little has been reported regarding the formation of inclusion complexes composed of amylose with high molecular weight guest compounds (polymeric guests) (Frampton et al., 2008; Ikeda et al., 2006; Kaneko, Kyutoku, Shimomura, & Kadokawa, 2011; Kida, Minabe, Nakano, & Akashi, 2008; Kida, Minabe, Okabe, & Akashi, 2007; Shogren, 1993; Shogren, Greene, & Wu, 1991). The principal difficulty for incorporating polymeric guests into the cavity of amylose is that the driving force for the complexation is only arising from the weak hydrophobic interaction. Amylose, therefore, does not have

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sufficient ability to include long chains of the polymeric guests into its cavity.

Over the past decade, we have developed the preparation method for such amylose-polymer inclusion complexes by means of the aforementioned phosphorylase-catalyzed enzymatic polymerization (Kadokawa, 2011, 2012; Kadokawa & Kaneko, 2013; Kadokawa & Kobayashi, 2010; Kaneko & Kadokawa, 2005). We have found that the propagation of the enzymatic polymerization proceeds with the formation of inclusion complexes when the phosphorylase-catalyzed polymerization was performed in the dispersion of appropriate hydrophobic polymers as guest compounds with aqueous buffer solutions as a polymerization solvent (Kadokawa, Kaneko, Nagase, Takahashi, & Tagaya, 2002; Kadokawa, Kaneko, Nakaya, & Tagaya, 2001; Kadokawa, Kaneko, Tagaya, & Chiba, 2001; Kadokawa, Nakaya, Kaneko, & Tagaya, 2003; Kaneko, Beppu, & Kadokawa, 2007; Kaneko, Beppu, & Kadokawa, 2008; Kaneko, Beppu, & Kadokawa, 2009; Kaneko, Saito, Nakaya, Kadokawa, & Tagaya, 2008; Kaneko, Beppu, Kyutoku, & Kadokawa, 2009; Kaneko, Ueno, Yui, Nakahara, & Kadokawa, 2011; Nomura, Kyotoku, Shimomura, Kaneko, & Kadokawa, 2011). The representation of this system is similar as the way that vines of plants grow twining around a rod. Accordingly, we have proposed that this polymerization method for the formation of amylose-polymer inclusion complexes is named 'vine-twining polymerization.'

By means of the vine-twining polymerization method using a designed polymeric guest compound, recently, we achieved to prepare a supramolecular hydrogel through the formation of inclusion complexes by amylose (Kaneko, Fujisaki, Kyutoku, Furukawa, & Kadokawa, 2010). In this study, we have designed a graft copolymeric guest compound, which forms network structures by the formation of intermolecular inclusion complexes with amylose by the vine-twining polymerization, giving rise to cross-linking points for the construction of hydrogels. Such a graft copolymer has to be soluble in water at the initial stage of the polymerization to act as a component of the hydrogel, whereas the hydrophobic nature is necessary for the graft chain structure to be included by amylose between the intermolecular graft copolymers. Taking these two antagonistic natures required for the graft copolymer structure into consideration, we have employed poly(acrylic acid sodium salt-*graft*-δ-valerolactone) (PAA-Na-*g*-PVL)) because PAA-Na has a hydrophilic nature to contribute to exhibiting water-solubility of the graft copolymer and PVL has been reported to be included by amylose in the vine-twining polymerization (Kadokawa, Nakaya, Kaneko, & Tagaya, 2003). When the vine-twining polymerization was performed in the presence of PAA-Na-*g*-PVL, the reaction mixture turned into the supramolecular hydrogel through the formation of inclusion complexes of amylose with the PVL graft chains in the intermolecular copolymers. We have continuously been investigating the extensive study on the construction of such polysaccharide supramolecular materials by means of the vine-twining polymerization.

In this paper, we report the preparation of new polysaccharide supramolecular films through the hydrogelation by the vine-twining polymerization (Scheme 1). To obtain the film form from the supramolecular hydrogels, we selected carboxymethyl cellulose (CMC) as the main-chain structure in a new guest graft copolymer in place of PAA-Na used in the aforementioned previous study (Kaneko, Fujisaki, Kyutoku, Furukawa, & Kadokawa, 2010) because of the film formability of CMC in addition to its water soluble nature (Feng, Pelton, & Ledue, 2006). Because CMC is one of the most well-known derivatives of cellulose, furthermore, it has been suitably employed as the component for the construction of polysaccharide supramolecular materials. We also replaced PVL of the hydrophobic graft chain employed in the previous study (Kaneko, Fujisaki, Kyutoku, Furukawa, & Kadokawa, 2010) by poly(ε-caprolactone) (PCL) because the latter is a more well-known biodegradable polyester than the former and was also found to be included by amylose by the vine-twining polymerization (Kadokawa, Kaneko, Nakaya et al., 2001). By the vine-twining polymerization in this study, consequently, the resulting films have been architected as the polysaccharide supramolecular structures composed of two representative natural polysaccharides, amylose and cellulose, inter-connected by the well-known biodegradable PCL (Scheme 1). Moreover, the present films exhibited the good mechanical properties under tensile mode, probably owing to incorporating the inclusion complex structures as supramoelcular cross-linking points. Therefore, we are convinced that the present films have highly potentials for applications to various practical fields as bio-based and eco-friendly materials.

2. Experimental part

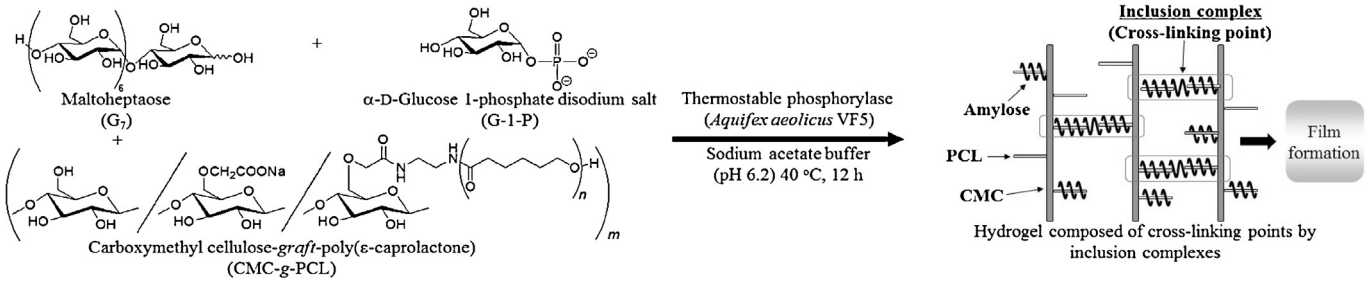
2.1. Materials and methods

Carboxymethyl cellulose (sodium salt, M_w = 700,000, Degree of carboxymethylation = 0.9) was purchased from Sigma–Aldrich Co. Phosphorylase from *Aquifex aeolicus* VF5 was supplied from Ezaki Glico Co. Ltd., Osaka, Japan (Bhuiyan, Rus'd, Kitaoka, & Hayashi, 2003; Yanase, Takata, Fujii, Takaha, & Kuriki, 2005; Yanase, Takaha, & Kuriki, 2006). Maltoheptaose (G_7) was prepared by selective cleavage of one glycosidic bond of β-cyclodextrin under acidic conditions (Braunmühl, Jonas, & Stadler, 1995). Poly(ε-caprolactone) (PCL) was prepared by ring-opening polymerization of ε-caprolactone initiated with 6-hydroxyhexanoic acid by scandium trifluoromethanesulfonate catalyst in toluene as described in the literature (Nomura, Taira, Tomioka, & Okada, 2000). 2-Azidoethylamine was synthesized by reaction of 2-bromoethylamine hydrobromide with sodium azide. A standard iodine-iodide solution was prepared according to the literature procedure (Kobayashi, Kamiya, & Enomoto, 1996). Other reagents and solvents were available commercially and used without further purification. ^{1}H NMR spectra were recorded on JEOL ECA 600 and JEOL ECX400 spectrometers. IR spectra were recorded on a SHIMADZU FTIR-8400 spectrometer. The powder X-ray diffraction (XRD) measurements were conducted using a PANalytical X'Pert Pro MPD with Ni-filtered CuKα radiation (λ = 0.15418 nm). The stress–strain curves were measured using a tensile tester (Little Senster LSC-1/30, Tokyo Testing Machine).

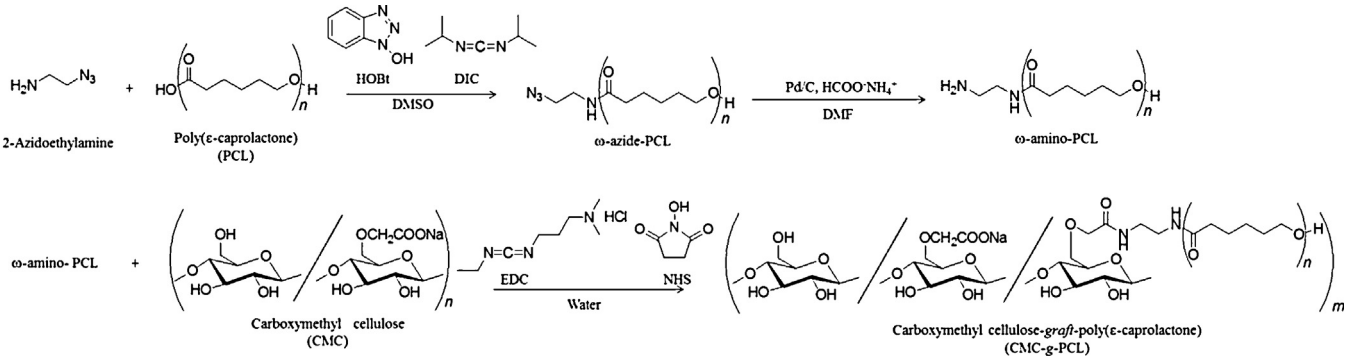
2.2. Synthesis of carboxymethyl cellulose-graft-poly(ε-caprolactone) (CMC-g-PCL) (Scheme 2)

To a solution of PCL (M_n = 660 by ^{1}H NMR, 0.500 g, 0.760 mmol) in DMSO (20 mL) was added 2-azidoethylamine (0.196 g, 2.28 mmol), 1-hydroxybenzotriazole(HOBt, 0.581 g, 3.80 mmol), and *N,N'*-diisopropylcarbodiimide (DIC, 0.480 g, 3.80 mmol) and the mixture was heated at 60 °C for 24 h with stirring. After the reaction mixture was diluted with chloroform, the solution was washed with water three times, dried over anhydrous Na_2SO_4, filtered, and concentrated. The concentrated solution was poured into a large amount of hexane to precipitate the product, which was isolated by decantation. This precipitation processes were repeated three times to give ω-azido-PCL (0.479 g) in 71.0% yield. ^{1}H NMR ($CDCl_3$): δ 1.39–1.43 (br, CH_2—C—C—C═O), 1.64–1.67 (br, CH_2—C—CH_2—C—C═O), 2.21 (t, CH_2—(C═O)—N, J = 7.5 Hz), 2.30–2.37 (br, CH_2—(C═O)—O), 3.40–3.45 (br, N_3—CH_2—CH_2—N), 3.65 (t, CH_2—OH, J = 6.1 Hz), 4.02–4.22 (br, O═C—C—C—C—C—CH_2—O).

To a solution of ω-azido-PCL (M_n = 890 by ^{1}H NMR, 0.355 g, 0.399 mmol) in DMF (3.5 mL) was added ammonium formate (0.126 g, 2.00 mmol) and 10% palladium on carbon (0.050 g) (Degée, Dubois, Jérôme, & Teyssié, 1992) and the mixture was stirred at



Scheme 1. Preparation of supramolecular hydrogel and film by vine-twining polymerization in the presence of CMC-*g*-PCL.



Scheme 2. Synthesis of CMC-*g*-PCL.

30 °C for 24 h. After powdered solids were removed by filtration, the filtrate was diluted with chloroform and the solution was washed with water three times, dried over anhydrous Na_2SO_4, filtered and concentrated. The concentrated solution was poured into a large amount of hexane to precipitate the product, which was isolated by decantation. These precipitation processes were repeated three times to give ω-amino-PCL (0.284 g) in 72.0% yield. ^{1}H NMR ($CDCl_3$): δ 1.39–1.43 (br, CH_2—C—C—C=O), 1.64–1.67 (br, CH_2—C—CH_2—C—C=O), 2.21 (t, CH_2—(C=O)—N, J = 7.5 Hz), 2.30–2.37 (br, CH_2—(C=O)—O), 2.84 (t, CH_2—NH_2, J = 5.8 Hz), (q, CH_2—NH=CO, J = 5.4 Hz), 3.65 (t, CH_2—OH, J = 6.8 Hz), 4.02–4.22 (br, O=C—C—C—C—C—CH_2—O); IR: 2106 cm^{-1} (—N_3).

To a dispersion of ω-amino-PCL (M_n = 990 by ^{1}H NMR, 0.100 g, 0.0950 mmol) in water (20 mL) was added CMC (0.227 g, 0.950 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 0.0182 g, 0.0950 mmol), and *N*-hydroxysuccinimide (NHS, 0.0109 g, 0.0950 mmol) and the mixture was stirred at room temperature for 24 h. The reaction mixture was poured into a large amount of methanol (300 mL) to precipitate the product, which was isolated by filtration and washed with methanol and chloroform. The product was dissolved in water and the solution was dialyzed in a dialysis bag (molecular weight cut off: 12,000–14,000) against water and lyophilized to give CMC-*g*-PCL (0.198 g). ^{1}H NMR (D_2O): δ 1.28–1.46 (br, CH_2—C—C—C=O), 1.46–1.70 (br, CH_2—C—CH_2—C—C=O), 2.14–2.42 (br, CH_2—(C=O)—O), 3.00–4.44 (br m, H_2-H_6 of CMC, O—CH_2—C=O, N—CH_2CH_2—N, O=C—C—C—C—C—CH_2—O), 4.44–4.68 (br, H_1 of CMC).

To determine the functionality of the graft chains, the PCL chains on CMC-*g*-PCL (0.0100 g) was hydrolyzed by treatment with 1.0 mol/L aqueous NaOH (2.0 mL) at room temperature for 6 h. The resulting solution was neutralized by 1.0 mol/L hydrochloric acid and dialyzed against water to give the hydrolyzed product. Then, the functionality was calculated by the integrated ratio of the signal due to O=C—C—CH_2—C—CH_2—C—O to the signal due to H_1 protons of CMC in the ^{1}H NMR spectrum of the hydrolyzed material to be 2.0%. In comparison of the integrated ratio with that before the alkaline hydrolysis, the average molecular weight of the PCL graft chains on CMC-*g*-PCL was estimated to be 1140.

2.3. Preparation of hydrogel by vine-twining polymerization in the presence of CMC-g-PCL

A typical experimental procedure for the preparation of hydrogel was as follows (Run 5, Table 1, G_7; 1.0 μmol, G-1-P/G_7 = 100). A mixture of CMC-*g*-PCL (0.0350 g) with 0.20 mol/L sodium acetate buffer (1.5 mL, pH = 6.2) was left standing at 40 °C for 30 min to give a homogeneous solution. Then, G-1-P disodium salt (0.304 g, 0.100 mmol), G_7 (0.0012 g, 1.0 μmol), and thermostable phosphorylase (9 U) was added to this solution and the solution was maintained at 40 °C for 12 h to give a hydrogel. To obtain a powdered sample, the resulting hydrogel was lyophilized, washed with DMSO, and subjected to filtration using a filter with 0.2 μm of the pore size. After the residue was dispersed with water and the dispersion was dialyzed in a dialysis bag (molecular weight cut off: 12,000–14,000) against water and lyophilized to yield the powdered sample (0.0416 g).

Table 1
Preparation of hydrogels by vine-twining polymerization in various feed amounts of G_7 and G-1-P/G_7 feed ratios and fracture stress and strain values of supramolecular films prepared from respective hydrogels under tensile mode[a]

Run	G_7 (μmol)	G-1-P/G_7	Fracture stress (MPa)	Fracture strain (%)
1	0.1	100	17	7
2	0.5	100	17	10
3	1.0	10	17	4
4	1.0	50	20	2
5	1.0	100	30	14
6	1.0	150	31	4
7	1.0	180	21	4
8	1.0	200	16	4
9	2.0	100	35	24
10	5.0	100	11	4

[a] Reaction conditions: CMC-*g*-PCL = 0.035 g, phosphorylase = 9 U, sodium acetate buffer = 1.5 mL, temperature = 40 °C, time = 12 h.

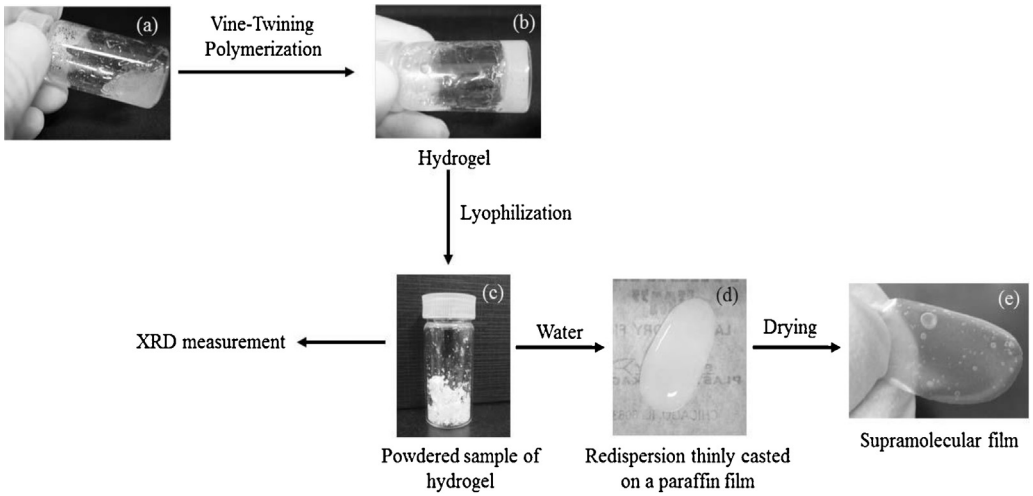


Fig. 1. Photographs before (a) and after (b) vine-twining polymerization, powdered sample (c), redispersion (d), and supramolecular film (e).

2.4. Preparation of supramolecular film

The powdered sample of the hydrogel (0.0400 g) was dispersed with water (1.5 mL). After the dispersion was thinly casted on a paraffin film, it was dried under the ambient atmosphere to produce a film.

2.5. Partial disruption of inclusion complexes in supramolecular film by guest exchange method

The supramolecular film (0.0350 g) was immersed in a standard iodide-iodine solution (1.3 mL). Then, the film was dried on a paraffin film under ambient atmosphere and subjected to tensile testing.

3. Results and discussion

3.1. Preparation of supramlecular hydrogels by vine-twining polymerization in the presence of carboxymethyl cellulose-graft-poly(ε-caprolactone)

For the synthesis of the guest graft copolymer, that is, CMC-*g*-PCL, according to Scheme 2, an amino group was introduced to a terminal end of PCL by the reaction of 2-azidoethylamine with a terminal carboxylic acid group using the HOBt/DIC condensing agent in DMSO, followed by the reduction to give ω-amino-PCL. Then, the condensation of ω-amino-PCL with carboxylate groups on CMC was performed using the EDC/NHS condensing agent in water to yield CMC-*g*-PCL. Taking the solubility of the product in water into account, the functionality and molecular weight of the PCL graft chain were adjusted to 2.0% and 1140, respectively.

The preparation of the hydrogel was performed by the phosphorylase-catalyzed enzymatic polymerization of G-1-P using G_7 as a primer (Run 5, Table 1) in the presence of the synthesized CMC-*g*-PCL in sodium acetate buffer (pH = 6.2) at 40 °C for 12 h according to the vine-twining polymerization manner (Scheme 1). The mixture turned into gelling form during the polymerization process (Fig. 1(a) → (b)). Because we assumed that the produced amylose formed inclusion complexes with the PCL graft chains between intermolecular (CMC-*g*-PCL)s during the propagation, which acted as cross-linking points, resulting in the formation of a hydrogel, the presence of such amylose-PCL inclusion complex structures in the product was confirmed by the XRD measurement of a powdered sample (Fig. 1(c)) prepared by lyophilization of the hydrogel. Consequently, the XRD pattern in Fig. 2(a) showed two diffraction peaks at 2θ = ca. 13 and 20°, which is similar as that of the inclusion complex of amylose with PCL as previously reported by us (Fig. 2(d)) (Kadokawa, Kaneko, Nakaya et al., 2001), but completely different from that of a sole amylose (Fig. 2(e)). These diffraction peaks have been reported to be ascribed to the helix diameters of ca. 1.3 nm corresponding to the amylose V complexes (Yamashita, 1965). Moreover, when the phosphorylase-catalyzed enzymatic polymerization was conducted in the presence of CMC in place of CMC-*g*-PCL, the produced amylose did not induce the formation of inclusion complexes with CMC (confirmed by the XRD result), and accordingly, the gelation of this system did not take place. These results supported the presence of inclusion complexes in the produced hydrogel, which could act as cross-linking points.

3.2. Formation of supramolecular films from hydrogels

For the preparation of a supramolecular film from the aforementioned hydrogel, the powdered sample for the XRD measurement was redispersed with water and the dispersion was thinly casted on a paraffin film (Fig. 1(d)). When the material was dried under ambient atmosphere, it formed the film as shown in Fig. 1(e). Accordingly, the several supramolecular films were prepared by the same procedure from the hydrogels composed of the different amounts of the amylose molecules with the same

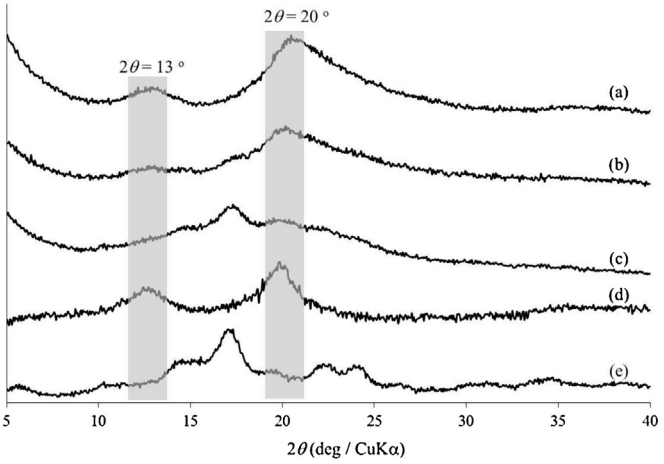


Fig. 2. XRD patterns of powered samples obtained from hydrogels of Run 5 (a), Run 9 (b), Run 10 (c) in Table 1, amylose-PCL inclusion complex (d), and amylose (e).

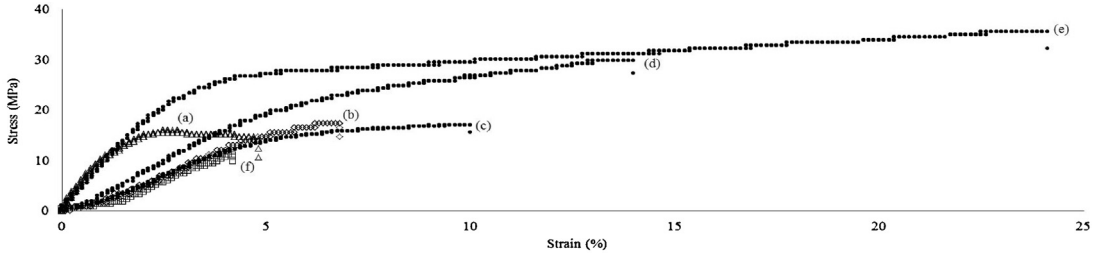


Fig. 3. Stress–strain curves of CMC film (a) and supramolecular films obtained from hydrogels of Runs 1 (b), 2 (c), 5 (d), 9 (e), and 10 (f) in Table 1 under tensile mode.

molecular weight, which were obtained by changing amounts of G_7 (0.1–5.0 μmol for 0.0350 g of CMC-*g*-PCL, Runs 1, 2, 5, 9, and 10, Table 1)) with the consistent G-1-P/G_7 feed ratio of 100 in the phosphorylase-catalyzed polymerization; the amount of G_7 and the G-1-P/G_7 feed ratio are representative factors to determine the amount and molecular weight of the produced amylose in the phosphorylase-catalyzed polymerization. The mechanical properties of the resulting films were then evaluated by tensile testing. As shown in Fig. 3, the mechanical properties were enhanced with initially increasing the amounts of the amylose molecules (Fig. 3(b) → (c)) and the films of Runs 5 and 9 (Fig. 3(d), (e)) showed much larger values in both fracture stress and strain compared with those of a CMC film (Fig. 3(a)). However, the mechanical property of the film with the larger amount of the amylose molecules (Run 10) became poor (Fig. 3(f)), probably because of the presence of the excess amylose molecules, which did not participate into the formation of inclusion complexes. Indeed, the XRD profiles of the films of Runs 5 and 9 showed the patters assigned to the inclusion complex (Fig. 2(a), (b)), whereas both the patterns due to the inclusion complex and amylose double helix were detected in the XRD profile of the film of Run 10 (Fig. 2(c)). These results suggested that the formation of double helixes from the excess amylose molecules caused the poor mechanical property.

The effect of the molecular weights of the amylose molecules in the films on the mechanical property was also evaluated. Therefore, the hydrogels composed of the consistent amounts of the amylose molecules with the different molecular weights were first prepared by the vine-twining polymerization in the different G-1-P/G_7 feed ratios, that is, 10–200, using the consistent amount of G_7 (G_7; 1.0 μmol for 0.0350 g of CMC-*g*-PCL, Runs 3–8, Table 1). Then, the films were prepared from the respective hydrogels by the same procedure as aforementioned. The stress–strain curves of these films by tensile testing in Fig. 4 indicated that the film of Run 5 (G-1-P/G_7 = 100) showed the good mechanical property with highly elastic nature (Fig. 4(c), the same data as that of Fig. 3(d)), whereas the other films composed of both the shorter and longer amylose chains (lower and higher molecular weights, respectively) exhibited the much lower fracture strain values, indicating their brittle nature. These results suggested the moderate amylose chain length was required to exhibit the elastic nature, probably for the efficient formation of inclusion complexes with the intermolecular (CMC-*g*-PCL)s during the vine-twining polymerization.

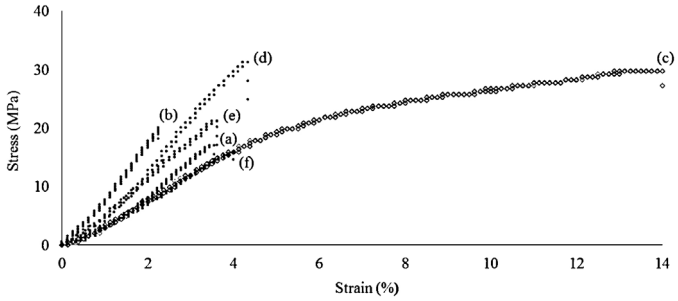


Fig. 4. Stress–strain curves of supramolecular films obtained from hydrogels of Runs 3–8 ((a)–(f), respectively) in Table 1.

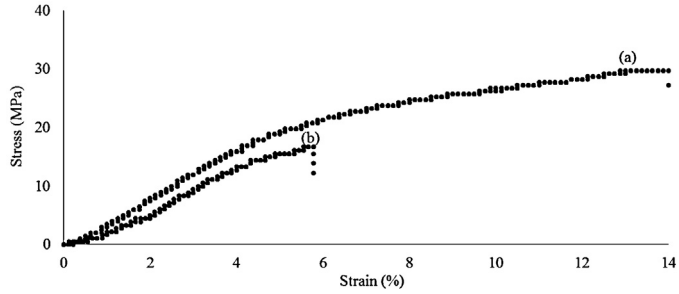


Fig. 5. Stress–strain curves of supramolecular film of Run 5 in Table 1 before (a) and after (b) treatment with standard iodine-iodide solution.

To confirm the effect of the supramolecular cross-linking on the mechanical property, the partial disruption of inclusion complexes in the film was conducted by the guest exchange approach. Because iodine has been a well-known guest molecule, which forms an inclusion complex with amylose (Kobayashi, Kamiya, & Enomoto, 1996), the present film (Run 5, Table 1) was immersed in a standard iodine-iodide solution. Then, the treated film was dried and subjected to tensile testing. The stress–strain curve of the treated film (Fig. 5(b)) showed the much lower fracture stress and strain values

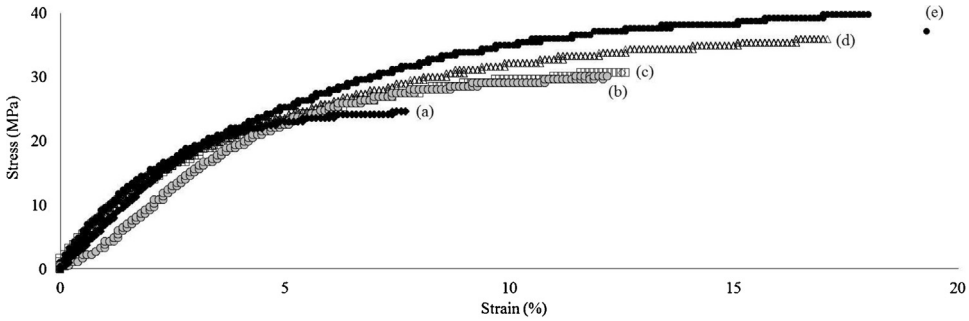


Fig. 6. Stress–strain curves by repeated tensions of supramolecular film of Run 5 in Table 1 (1st–5th cycles, (a)–(e), respectively).

than those before treatment (Fig. 5(a)), indicating that the presence of the amylose-PCL graft chain inclusion complexes as cross-linking points strongly affected the enhancement of the mechanical property. We also found that the present supramolecular film exhibited the further unique mechanical property as follows. The film (Run 5, Table 1) was tensioned and relaxed before fracture. When the tensile testing of the film was then conducted after the processes were repeated four times (Fig. 6(a) → (d)), the stress–strain curve (Fig. 6(e)) showed the larger fracture stress and strain values than those of the original film (Fig. 3(d)). Although the detailed reason for exhibiting such unique mechanical property is not yet clear, this property is probably owing to the supramolecular cross-linking structure in the film.

4. Conclusions

In this study, we investigated the preparation of amylose supramolecular films through the hydrogelation by the vine-twining polymerization using the new guest graft copolymer. For this purpose, we designed and synthesized CMC-*g*-PCL, and the hydrogels were prepared according to the vine-twining polymerization procedure using this graft copolymer. Because we supposed that amylose included the PCL graft-chains in the intermolecular (CMC-*g*-PCL)s to produce cross-linking points for the hydrogelation during the polymerization, the presence of inclusion complexes composed of amylose and the PCL graft-chains was confirmed by the XRD measurement of the powdered sample prepared by lyophilization of the hydrogel. Then, the films were obtained by adding water to the powdered samples, followed by drying. The mechanical properties of the resulting films composed of the appropriate amounts of the amylose molecules with the moderate molecular weights were superior to those of a CMC film. The supramolecular cross-linking structure in the films probably contributed to enhancing the mechanical property. These results indicate that the present films are expected to be used in practical applications as eco-friendly and functional bio-based materials in the future.

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