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.

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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 $\alpha(1\rightarrow4)$-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 $\alpha(1\rightarrow4)$-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