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Preparation of Multiformable Supramolecular Gels through Helical Complexation by Amylose in Vine-Twining Polymerization

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In this study, we prepared mutiformable functional supramolecular gels by vine-twining polymerization using poly(γ-glutamic acid-*graft*-ε-caprolactone) (PGA-*g*-PCL) as a new guest polymer, with subsequent procedures of lyophilization and exchange of dispersion media. When the phosphorylase-catalyzed enzymatic polymerization of the monomer α-D-glucose 1-phosphate from a maltoheptaose primer was carried out in the presence of PGA-*g*-PCL according to the vine-twining polymerization method, a supramolecular hydrogel was obtained. The resulting hydrogel, purified by soaking in water, had the self-standing property. Macroscopic interfacial healing was achieved by the formation of inclusion complexes at the interface between two hydrogel pieces through enzymatic polymerization. Cryogels were obtained by the lyophilization of the hydrogels; XRD analysis of the cryogel indicated the presence of inclusion complexes of amylose with PCL graft chains in intermolecular (PGA-*g*-PCL)s, which acted as cross-linking points for hydrogelation. Porous morphologies were seen in scanning electron micrographs of the cryogels. Furthermore, ion gels were fabricated by soaking the hydrogels in the ionic liquid of 1-butyl-3-methylimidazolium chloride. The mechanical properties of the cryo- and ion gels were evaluated by compressive and tensile testing, respectively.

Introduction

Biological macromolecules such as polysaccharides, proteins, and nucleic acids appear in the important *in vivo* functions associated with living.[1, 2] The vital functions of such macromolecules appear to result not only from the specific primary structures of the molecules but also by the construction of regular higher-order structures based on controlled non-covalent linkages, representatively demonstrated by the double helix of the DNA.[3] The research field concerning the precision architecture of hierarchically fabricated higher-order structures from synthetic macromolecules, so-called "supramolecular chemistry," has been of increasing importance from the viewpoint of the corroboration of biological systems.[4-6] Among the wide variety of supramolecules, host-guest materials are one of the most extensively studied. In these pairings, host compounds interact with guest compounds through complexation by non-covalent linkages.[7-9] As host compounds, cyclic molecules such as crown ether, cyclodextrin, and calixarene are well-known to form host-guest supramolecules, known as inclusion complexes, with the appropriate guest compounds.[10]

Amylose, composed of α-(1→4)-linked D-glucose residues, is a natural polysaccharide and a component of starch, as well as a well-known host compound because of the left-handed helical conformation of the molecule with a hydrophobic field within the cavity of the helix.[11] Therefore, it forms host-guest inclusion complexes with various guest compounds of suitable geometrical structures and molecular sizes, typically monomeric and oligomeric compounds, by hydrophobic interactions.[12] However, little has been reported regarding the formation of inclusion complexes composed of amylose with high-molecular-weight polymeric guest compounds.[13-22] The principal difficulty for the incorporation of polymeric guests into the cavity of amylose is the relatively weak driving force for the complexation arising solely from hydrophobic interactions. Therefore, it can be considered that amylose may not have sufficient capacity to directly include long chains of polymeric guests into the cavity of the host molecule.

Over the past decade, we have developed a method for the construction of such amylose-polymer inclusion complexes by means of enzymatic polymerization catalyzed by phosphorylase, which forms structurally well-defined amylose.[23] The phosphorylase-catalyzed enzymatic polymerization is conducted using α-D-glucose 1-phosphate (G-1-P) as a monomer and is initiated from the non-reducing end of a maltooligosaccharide primer, such as maltoheptaose (G_7). The propagation proceeds through the following reversible reaction to produce amylose: $[\alpha\text{-}(1\rightarrow4)\text{-G}]_n$ + G-1-P ⇄ $[\alpha\text{-}(1\rightarrow4)\text{-G}]_{n+1}$ + Pi.[24-27] In the reaction, D-glucose units are successively transferred from G-1-P to the non-reducing 4-OH propagating end of a α-(1→4)-glucan chain, leading to chain elongation accompanied by the liberation of inorganic phosphates (Pi). We have found that the propagation of the phosphorylase-catalyzed enzymatic polymerization proceeded with the formation of inclusion complexes when the

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polymerization was performed in a dispersion of appropriate hydrophobic polymers as guest compounds, such as the well-known biodegradable polyester poly(ε-caprolactone) (PCL) with an aqueous buffer solution as a polymerization solvent.[28, 29] A geometrical representation of this system is similar to the way that plant vines grow to twine around a supporting rod. Accordingly, this polymerization method for the formation of amylose-polymer inclusion complexes has been named "vine-twining polymerization."[30-35]

Recently, by means of the vine-twining polymerization approach using designed polymeric guests, we have achieved the fabrication of supramolecular hydrogels through inclusion complexation by amylose. In these studies, we employed graft copolymers, such as poly(acrylic acid sodium salt-*graft*-δ-valerolactone) (PAA-*g*-PVL) and carboxymethyl cellulose-*graft*-PCL) (CMC-*g*-PCL), as the designed guest polymers.[36, 37] The hydrophilic main chains of PAA and CMC acted as components in the hydrogels, whereas the hydrophobic graft polyesters of PVL and PCL were found to behave as guest polymers for inclusion complexation by amylose in the vine-twining polymerization.[28, 29] When the vine-twining polymerization was performed using such guest graft copolymers, the reaction mixture formed supramolecular hydrogels through the formation of inclusion complexes of amylose with the graft chains in the intermolecular copolymers. The intermolecular inclusion complexes acted as cross-linking points to form host-guest network structures in the hydrogels. Because the mechanical properties of the resulting hydrogels were very weak, the hydrogels could not be investigated further regarding the characterization of the possible functions and practical material applications. We have continuously conducted extensive studies on the construction of more mechanically robust supramolecular hydrogels in other systems utilizing vine-twining polymerization.

In this study, we found that self-standing supramolecular hydrogels were obtained when poly(γ-glutamic acid) (PGA), a natural polypeptide, was employed as the main chain in a new guest graft copolymer with PCL graft chains (poly(γ-glutamic acid-*graft*-ε-caprolactone) (PGA-*g*-PCL)) in the vine-twining polymerization method. Furthermore, additional investigations revealed that the obtained hydrogel exhibited macroscopic interfacial healing behavior through enzymatic polymerization and could be further converted into other supramolecular gel materials, such as cryo- and ion gels, by the appropriate procedures. Because the present multiformable supramolecular gels showed highly functional performances, and because they are totally composed of bio-related polymeric components, they have the potential for practical applications as biomedical and environmental materials.

Results and Discussion

Preparation of supramolecular hydrogels by vine-twining polymerization

For the synthesis of the guest graft copolymer PGA-*g*-PCL, an amino group was introduced to a terminal carboxyl end of PCL by a reaction with 2-azidoethylamine, using the 1-hydroxybenzotriazole/*N,N'*-diisopropylcarbodiimide condensing agent in dimethyl sulfoxide (DMSO), followed by catalytic hydrogenation, according to the procedure previously reported by us, to produce ω-amino-PCL.[37] The condensation of the product with carboxylate groups on PGA was conducted, using the condensing agent of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) in water/DMSO to produce PGA-*g*-PCL (Fig. 1a). The structure of the product was confirmed by a ^{1}H NMR spectrum in D_2O (Fig. S1). Accounting for the solubility of PGA-*g*-PCL in water, the degree of functionalization, and the molecular weight of the PCL graft chain, estimated by the integrated ratio of the signals corresponding to the terminal protons of the PCL graft chain connected to the PGA main-chain to the signals corresponding to PGA and PCL protons, were adjusted to relatively low values of 2.8% and 1040, respectively.

The preparation of the supramolecular hydrogel was performed by the phosphorylase-catalyzed enzymatic polymerization of G-1-P from a G_7 primer in the presence of PGA-*g*-PCL in a sodium acetate buffer (pH = 6.2) at 40 °C for 15 h, according to the vine-twining polymerization method (Fig. 1b). The transparent reaction solution gradually turned into a gelled form as the polymerization progressed (Fig. 1c (I→II)). The resulting hydrogel was soaked in water three times (10 min each) to remove the unreacted G-1-P, enzyme, and buffer salt for purification. The preparation of the hydrogels was conducted in this manner under the different enzymatic polymerization conditions presented in Table 1. The number of amylose molecules, produced by the enzymatic polymerization, was changed depending on the amount of G_7 primer (runs 1-3), whereas the molecular weight of amylose was maintained at a constant value by using the same G-1-P/G_7 molar ratio (= 100) in all cases. Consequently, all hydrogels obtained under the conditions of runs 1-3 exhibit self-standing properties (Fig. 1c (III)), indicating the appearance of much improved mechanical properties of the present materials by the use of PGA as the main chain, compared to the previous hydrogels obtained using PAA-*g*-PVL and CMC-*g*-PCL. One of the conceivable reasons for the production of the more robust hydrogels is probably due to the high water retention and moisturizing properties of PGA.[38]

The resulting hydrogels were characterized by oscillatory shear rheometry to determine the dynamic viscoelastic properties of the materials. The frequency dependence of the storage and loss moduli (*G'* and *G"*, respectively) in Fig. 2 shows the signature behavior of typical viscoelastic materials, with the predominance of the storage modulus throughout the frequency range. These results support the gelling state of all hydrogels from runs 1-3. Furthermore, the differences between the *G'* and *G"* values increase with increasing the amount of G_7 (run 1 → run 3, Fig. 2a → 2c), probably because of the increased cross-linking densities by the formation of larger numbers of inclusion complexes in this order of increasing the amount of G_7. Indeed, the water contents of the hydrogels, calculated by the measured weight difference in the

materials after lyophilization, are observed to decrease with increasing amounts of G_7, as shown in Table 1.

The above results suggest that the enzymatically produced amylose formed inclusion complexes with the PCL graft chains in intermolecular (PGA-*g*-PCL)s during the enzymatic polymerization, which acted as cross-linking points, resulting in hydrogelation. The presence of such amylose-PCL inclusion complex structures in the hydrogel, therefore, was confirmed by powder X-ray diffraction (XRD) measurement of a powdered sample (cryogel, *vide infra*) prepared by the lyophilization of the hydrogel (run 3). The XRD profile in Fig. 3a shows two obvious diffraction peaks at 2θ = *ca.* 13 and 20°, a typical pattern for the inclusion complex from amylose, as observed in that of the amylose-PCL inclusion complex, as we previously reported (Fig. 3b).[28, 29] However, the pattern is completely different from that of PGA-*g*-PCL, enzymatically synthesized amylose by phosphorylase, and PCL (Fig. 3c-e). The XRD profiles of the other samples (runs 1 and 2, Fig. S2) also showed similar diffraction patterns to those shown in Fig. 3b, although the peak intensities of the patterns from the lyophilized samples of runs 1-3 were lower, because of the lower amounts of inclusion complexes compared to the bulk amylose-PCL inclusion complex. This XRD result strongly supports the presence of inclusion complexes in the hydrogel; these complexes could act as cross-linking points for hydrogelation.

Macroscopic interfacial healing of hydrogels through enzymatic polymerization

We have already revealed that the supramolecular hydrogel composed of PAA-*g*-PVL exhibited enzymatically disruption-reconstruction behavior on the molecular level.[36] The gel was disrupted by the β-amylase-catalyzed hydrolysis of the amylose component in the inclusion complexes, and the resulting solution returned to the hydrogel form by the reformation of inclusion complexes via in-situ phosphorylase-catalyzed enzymatic polymerization. It has been also demonstrated that host and guest gels composed of the cyclodextrin-interaction system show macroscopic interfacial self-assembly through molecular recognition to form larger aggregated structures.[39-48] Because the present hydrogel is composed of supramolecular networks by the cross-linked host-guest inclusion complexation of amylose with the PCL graft chains, macroscopic interfacial healing through the phosphorylase-catalyzed enzymatic polymerization has been successfully demonstrated. The hydrogel (run 3) was cut into two pieces. A sodium acetate buffer solution containing phosphorylase and G-1-P (the enzymatic polymerization solution) was applied to the surfaces of the two hydrogel pieces. After the surfaces were placed in contact, the gels were left standing at 40 °C for 6 h for the progress of the polymerization. After the elapsed time, the two pieces had cohered at the interface (Fig. 4a). In contrast, when the same procedure was conducted using a sodium acetate buffer without the enzyme and monomer, the surfaces were not cohered at all (Fig. 4b). The firm adhesion was further confirmed by tensile testing. The adhered hydrogel exhibited an elongation value of 28.6% at break, comparable to the value of an original hydrogel (28.0%). These results indicate that the healing of the gels on a macroscopic level was achieved by the complexation of the enzymatically produced amylose molecules with the PCL graft chains at the interface (Fig. 4). This macroscopic interfacial healing behavior can be considered to mimic the process of self-healing by in vivo enzymatic reactions in tissues.

Conversion into supramolecular cryogel and ion gel

The supramolecular hydrogels were successfully converted into other supramolecular gels by the appropriate procedures. An attempt was made to convert the hydrogels into supramolecular cryogels by lyophilization (Fig. 5a). The scanning electron microscopy (SEM) images of the produced materials from the hydrogels of runs 1-3 show porous morphologies (Fig. 6). Furthermore, the pore size decreases with increasing the amount of G_7 for hydrogelation (Fig. 6a,b → 6c,d → 6e,f), owing to the formation of smaller networks with higher cross-linking densities with larger amounts of G_7. The stress-strain curves of the cryogels under compressive testing indicate that the pore size affects the mechanical properties. The cryogel with the smallest pore sizes (from the hydrogel of run 3) shows the hardest nature with the largest fracture stress value and the smallest fracture strain value (Fig. 7a-c). To estimate the molecular weight (M_n) of amylose, the cryogel (run 3) was treated with 1.0 mol/L aqueous NaOH to hydrolyze the PCL graft chains. After neutralization by aqueous HCl, the precipitated amylose was isolated by filtration, washed with water, acetone, and chloroform, and dried under reduced pressure. The M_n value of the isolated amylose was estimated to be 16500 by measuring the value of λ_{max} in the UV-vis spectrum after complexation with iodine.[49, 50] Re-hydrogelation of the cryogel was achieved by soaking the cryogel in water for 3 h. The dynamic viscoelastic data of the resulting hydrogel was comparable to that of the original (run 3), suggesting good re-hydrogelation behavior of the obtained cryogel.

The conversion into supramolecular ion gels through the exchange of dispersion media was also demonstrated by soaking the hydrogels in the ionic liquid of 1-butyl-3-methylimidazolium chloride (BMIMCl) at room temperature for 24 h (Fig. 5b). BMIMCl is well-known to be a good solvent of polysaccharides.[51, 52] The sufficient exchange of dispersion media was confirmed by thermogravimetric analysis (TGA), in which the TGA curve of the resulting ion gel showed no obvious weight loss at temperatures up to 100 °C; this indicated that no evaporation of water occurred. The stress-strain curves of the ion gels under tensile testing show that the values of both tensile strength and elongation at break increase with increasing the amount of G_7 for the hydrogelation process (Fig. 7d-f). This suggests that the mechanical properties of the ion gels is strongly affected by the cross-linking density, and consequently, the ion gel from the hydrogel of run 3 shows good mechanical properties with 21.0% elongation at break.

Conclusions

Here, we reported the preparation and characterization of supramolecular hydro-, cryo-, and ion gels by the vine-twining polymerization method using PGA-*g*-PCL. The resulting hydrogels exhibited self-standing properties. The hydrogels also showed macroscopic interfacial healing behavior by the inclusion complexation by amylose at the interface, through phosphorylase-catalyzed enzymatic polymerization. The XRD results of the cryogel obtained by the lyophilization of the hydrogel indicated the presence of inclusion complexes of amylose with the PCL graft chains in the intermolecular graft copolymers, which acted as cross-linking points for the hydrogelation. The SEM images of the cryogels revealed porous morphologies. Soaking the hydrogels in BMIMCl induced an exchange of dispersion media to produce the ion gels. The obtained supramolecular gels are composed of bio-related polymeric components, such as natural polysaccharide, natural polypeptide, and biodegradable polyester, and exhibited macroscopic interfacial healing behaviors. Accordingly, they have the potential for practical material applications in biomedical and environmental fields in the future.

Experimental section

Instruments and methods

^{1}H NMR spectra were recorded on JEOL ECA 600 and JEOL ECX400 spectrometers. The dynamic viscoelastic measurement was conducted on a rheometer (Rheosol-G1000, UBM). The SEM images were obtained using a Hitachi S-4100H electron microscope. The XRD measurement was 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 Senstar LSC-1/30, Tokyo Testing Machine). TGA measurement was performed on an SII TG/DTA 6200 at a heating rate of 10 °C min^{-1}. The UV-vis measurement was conducted using a Jasco V-650Q1 spectrometer.

Materials

PGA (M.W. = 1.5 – 2.5 x 10^6) was purchased from Wako Pure Chemicals, Tokyo, Japan. Phosphorylase from *Aquifex aeolicus* VF5 was supplied from Ezaki Glico Co. Ltd., Osaka, Japan.[26, 53, 54] A primer, G_7, was prepared by the selective cleavage of one glycosidic bond of β-cyclodextrin under acidic conditions.[55] ω-Amino-PCL was synthesized according to the literature procedure.[37] Other reagents and solvents were available commercially and used without further purification.

Synthesis of PGA-*g*-PCL

A solution of ω-amino-PCL (M_n = 1100, 0.0782 g, 0.0713 mmol) in DMSO (7.0 mL) was mixed with a solution of PGA (0.129 g, 1.00 mmol) in 0.3 mol/L aqueous NaOH (3.0 mL) at room temperature to produce a homogeneous solution. After EDC (0.0192 g, 0.100 mmol) and NHS (0.115 g, 1.00 mmol) were added to the solution at room temperature, the mixture was stirred at 60 °C for 24 h. The reaction solution was then lyophilized and chloroform (40 mL) was added to the residue. After the mixture was stirred at room temperature for 3 h, the insoluble fraction was isolated by filtration and dried under reduced pressure to yield the crude PGA-*g*-PCL. The product was then purified by dialysis against water (molecular weight cut-off: 1000) and lyophilized to yield PGA-*g*-PCL (0.162 g, 0.896 mmol) with an 89.6% yield. ^{1}H NMR (D_2O): δ 1.32-1.43 (br, CH_2-C-C-C=O), 1.52-1.70 (br, CH_2-C-CH_2-C-C=O), 1.93-2.02, 2.06-2.18 (br, β-CH_2 of PGA), 2.20-2.25 (br, CH_2-(C=O)-N), 2.31-2.45 (br, γ-CH_2 of PGA, CH_2-(C=O)-O), 3.26-3.40 (br, N-CH_2CH_2-N), 3.54-3.62 (br, C*H*$_2$-OH), 4.05-4.16 (br, CH_2-OC=O), 4.17-4.29 (br, α-CH of PGA).

Preparation of supramolecular hydrogels by vine-twining polymerization and conversion into cryogels

A typical experimental procedure for the preparation of hydrogel was as follows (run 3, Table 1). A mixture of PGA-*g*-PCL (0.040 g, 0.221 mmol) with 0.20 mol/L sodium acetate buffer (2.0 mL, pH = 6.2) was left standing at room temperature for 5 h to produce a homogeneous solution. G-1-P disodium salt (0.187 g, 0.616 mmol), G_7 (0.0071 g, 6.16 μmol), and thermostable phosphorylase (9 U) were added to this solution and the mixture was maintained at 40 °C for 15 h to yield a hydrogel. The resulting hydrogel was soaked in water three times (10 mL, 10 min each) to yield the purified hydrogel. The hydrogel was lyophilized to produce a cryogel. The water content of the hydrogel was estimated, by the weight difference between the cryogel and the hydrogel, to be 84.2%.

Macroscopic interfacial healing of hydrogels through enzymatic polymerization

A solution of G-1-P (0.0047 g, 15.5 mmol) and thermostable phosphorylase (1 U) in a sodium acetate buffer (50 μL, pH = 6.2) was applied to the surfaces of two hydrogel pieces (run 3). After the surfaces were placed in contact, the material was left standing at 40 °C for 6 h. As a control experiment, sodium acetate buffer alone was applied to the surfaces of two hydrogel pieces and the subsequent procedure was similarly conducted.

Preparation of ion gels by exchange of dispersion media

Rectangular hydrogels (*ca.* 10 × 20 × 5 mm) were first prepared by the vine-twining polymerization according to the conditions of runs 1-3. The resulting hydrogels were soaked in BMIMCl (2.50 g) at room temperature for 24 h. The products were dried under reduced pressure to produce ion gels.

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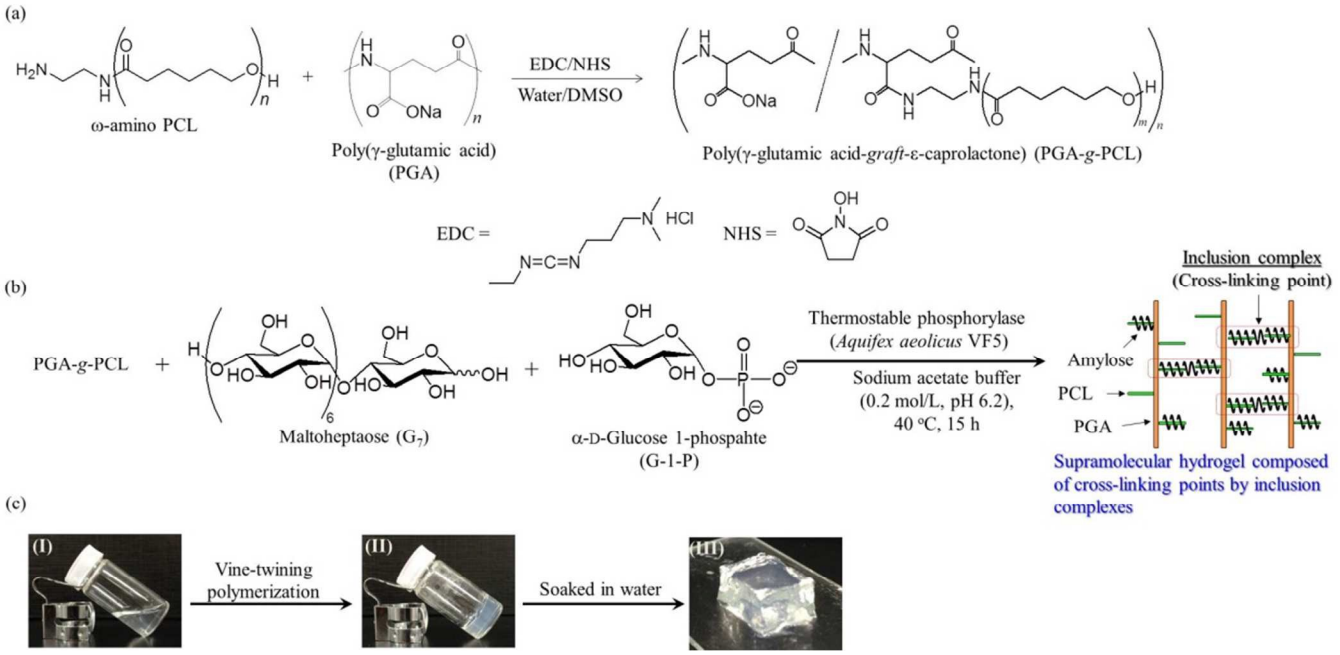

Fig. 1 Synthesis of poly(γ-glutamic acid-*graft*-ε-caprolactone) (PGA-*g*-PCL) (a), preparation of supramolecular hydrogel by vine-twining polymerization using PGA-*g*-PCL (b), and photographs before (a) and after (b) vine-twining polymerization and purified supramolecular hydrogel (c).

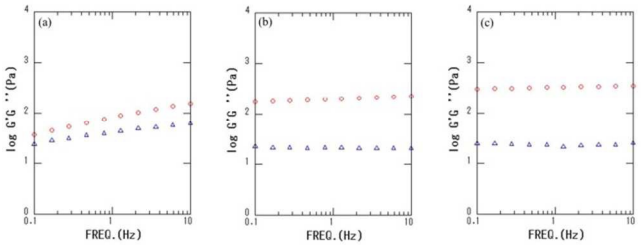

Fig. 2 Evaluation of storage modulus G' (circles) and loss modulus G" (triangles) as a function of frequency for supramolecular hydrogels of runs 1-3 (a, b and c, respectively).

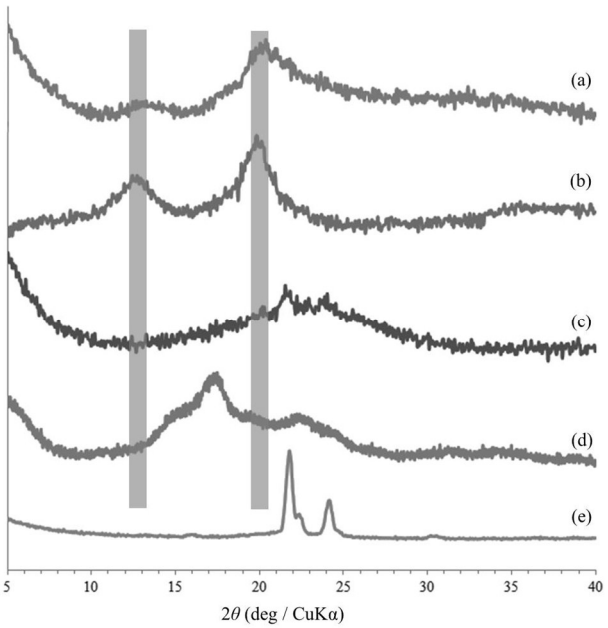

Fig. 3 XRD patterns of lyophilized sample from supramolecular hydrogel of run 3 (a), amylose-PCL inclusion complex (b), PGA-*g*-PCL (c), enzymatically synthesized amylose (d), and PCL (e).

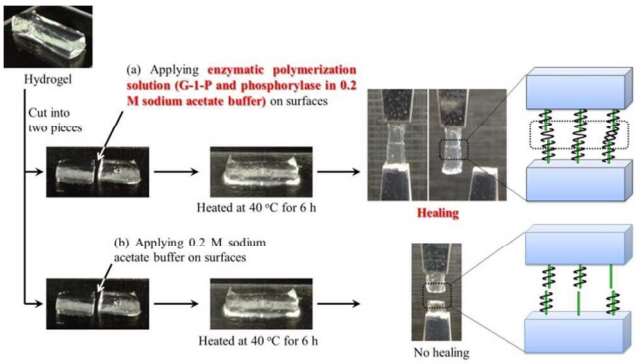

Fig. 4 Macroscopic interfacial healing experiment through enzymatic polymerization (a) and control experiment (b).

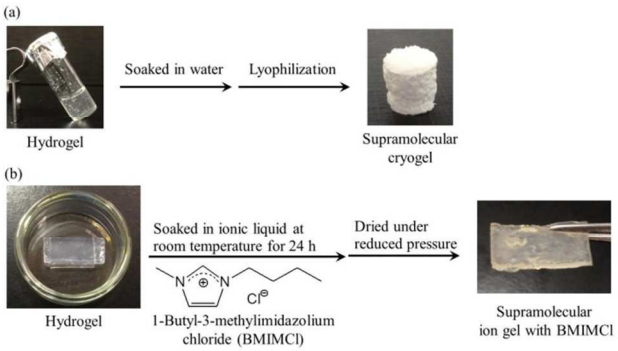

Fig. 5 Conversion of supramolecular hydrogel into cryogel (a) and ion gel (b).

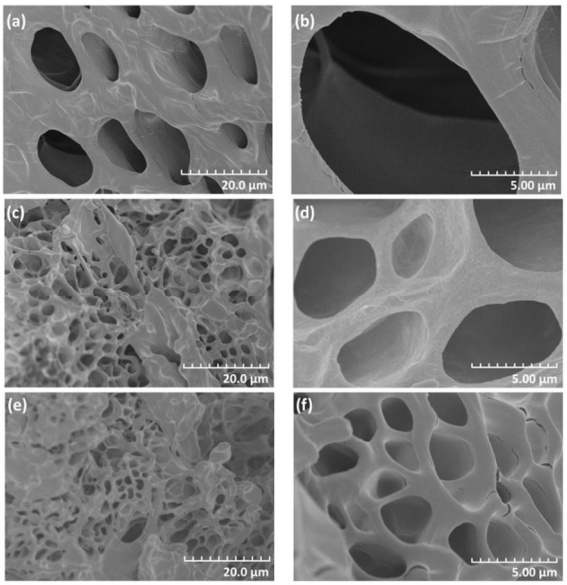

Fig. 6 SEM images of cryogels obtained from supramolecular hydrogels of runs 1 (a, b), 2 (c, d), and 3 (e, f).

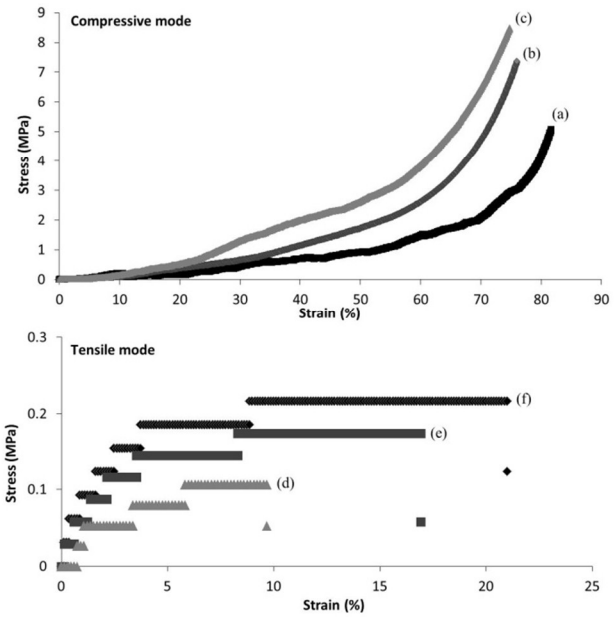

Fig. 7 Stress-strain curves of cryogels under compressive mode and ion gels under tensile mode obtained from supramolecular hydrogels of runs 1 (a, d), 2 (b, e), and 3 (c, f).

Table 1 Preparation of supramolecular hydrogel and characterization of hydro-, cryo-, and ion gels

Run	Vine-twining polymerization[a]		Water content of hydrogel[b]	Mechanical property of cryogel under compressive mode		Mechanical property of ion gel under tensile mode	
	G_7 (μmol)	G-1-P (mmol)	(%)	Fracture stress (MPa)	Fracture strain (%)	Tensile strength (MPa)	Elongation at break (%)
1	0.868	0.087	94.2	5.1	81.6	0.11	9.7
2	3.73	0.373	87.4	7.4	75.9	0.17	16.9
3	6.16	0.616	84.2	8.5	74.7	0.22	21.0

[a] Reaction was carried out in G-1-P/G_7 feed molar ratio = 100 using PGA-*g*-PCL (0.0400 g, 0.221 mmol) in the presence of thermostable phosphorylase (9 U) in 0.2 mol/L sodium acetate buffer (2.0 mL, pH = 6.2) at 40 °C for 15 h. [b] Water content was estimated by the weight difference of cryogel from hydrogel.

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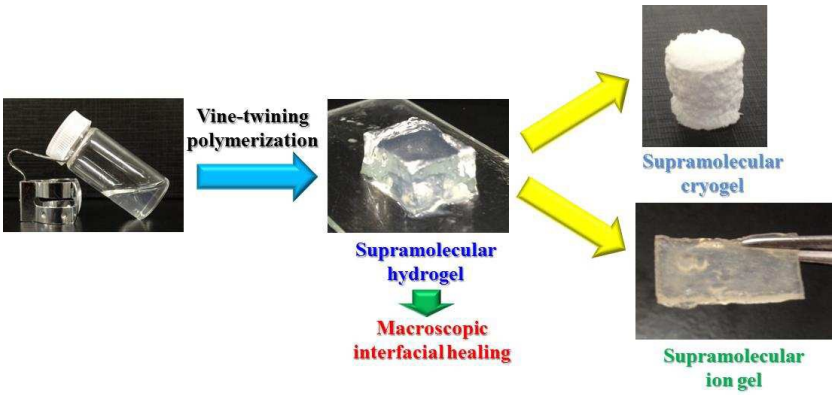
Preparation of Multiformable Supramolecular Gels through Helical Complexation by Amylose in Vine-Twining Polymerization

Jun-ichi Kadokawa, Kazuya Tanaka, Daisuke Hatanaka, Kazuya Yamamoto



Supramolecular hydrogels with macroscopic interfacial healing behavior were successfully obtained through helical complexation by amylose in the vine-twining polymerization using poly(γ-glutamic acid-*graft*-ε-caprolactone), which were further converted into supramolecular cryo- and ion gels.