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Low-Temperature Processable Block Copolymers That Preserve the Function of Blended Proteins

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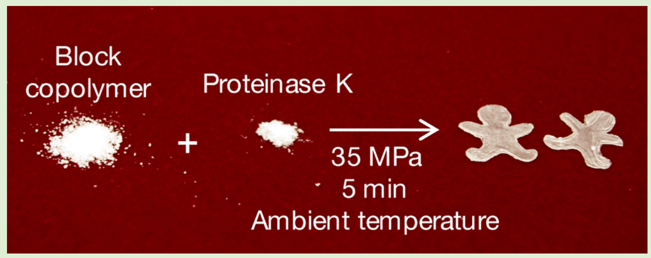
Supporting Information



ABSTRACT: Low-temperature processable polymers have attracted increasing interest as ecological materials because of their reduced energy consumption during processing and suitability for making composites with heat-sensitive biomolecules at ambient temperature. In the current study, low-temperature processable biodegradable block copolymers were synthesized by ring-opening polymerization of L-lactide (LLA) using polyphosphoester as a macroinitiator. The polymer films could be processed under a hydraulic pressure of 35 MPa. The block copolymer films swelled in water because the polyphosphoester block was partially hydrated. Interestingly, the swelling ratio of the films changed with temperature. The pressure-induced order-to-disorder transition of the block copolymers was characterized by small-angle X-ray scattering; a crystallinity reduction in the block copolymers was observed after application of pressure. The crystallinity of the block copolymers was recovered after removing the applied pressure. The Young's modulus of the block copolymer films increased as the LLA unit content increased. Moreover, the modulus did not change after multiple processing cycles and the recyclability of the block copolymers was also confirmed. Finally, polymer films with embedded proteinase K as a model protein were prepared. The activity of catalase loaded into the polymer films was evaluated after processing at different temperatures. The activity of catalase was preserved when the polymer films were processed at room temperature but was significantly reduced after high-temperature processing. The suitability of low-temperature processable biodegradable polymers for making biofunctional composites without reducing protein activity was clarified. These materials will be useful for biomedical and therapeutic applications.

INTRODUCTION

Composite materials comprising biodegradable polymer matrices and biomolecules have recently become attractive materials in both biomedical and tissue engineering fields.[1−4] Typical biodegradable polymers, such as aliphatic polyesters, show thermoplastic behavior and are normally processed by melt-molding while heating. However, the thermal properties of aliphatic polyesters are relatively poor.[5,6] Moreover, the deactivation of biomolecules in composite materials by heat treatment is inevitable. Several techniques, such as solution casting, electrospinning, and emulsion, have been proposed to fabricate the composite materials without heat treatment.[7−9] However, these approaches often induce denaturation of biomolecules because typical aliphatic polyesters are insoluble in aqueous media so organic solvents have to be used. Furthermore, these alternative processes yield inferior processability compared with melt-molding.

As an alternative to hot-melt processing, Pollard et al. reported that block copolymers comprising a certain pair of low glass-transition temperature (T_g) and high T_g blocks could be processed at temperatures as low as room temperature under pressure.[10] Such materials are termed "baroplastics" because of their ability to transform from a solid to a melt state via an order-to-disorder phase transition induced by the application of pressure.[11−14]

Taniguchi and Lovell first reported low-temperature processable biodegradable polymers, which possess low-T_g poly(ε-caprolactone) (PCL) derivatives and high-T_g poly(L-lactide) (PLLA).[15] The block copolymers show room-temperature processability under a hydraulic pressure of 34.5 MPa without polymer degradation. The pressure-induced phase transition of the block copolymers was determined by small-angle X-ray scattering (SAXS). Moreover, the mechanical properties of processed specimens at low temperature could be controlled by changing the ratio of the soft to hard segments. Although the combination of PCL derivatives and PLLA endows the resulting block copolymers with pressure processability, the variety of biodegradable baroplastics is still

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limited. Furthermore, composite materials comprising baroplastics and biomolecules have not yet been proposed.

In the current study, we propose new types of biodegradable baroplastics comprising polyphosphoester and PLLA. Polyphosphoesters have made a large impact in biorelated fields because of their biocompatibility and structural similarity to naturally occurring nucleic acids.[16−18] Compared with conventional aliphatic polyesters, the molecular functionalization of polyphosphoesters is easier because various cyclic phosphoesters, which work as monomers, can be obtained by a simple condensation reaction between an alcohol and 2-chloro-2-oxo-1,3,2-dioxaphosphorane (COP).[19,20] That is, theoretically, any alcohol can be introduced into polyphosphoesters. Furthermore, the solubility of polyphosphoesters can be controlled by the substituent groups on the side chain. We have successfully polymerized cyclic phosphoester monomers using organocatalysts.[21] The advantage of the organocatalysts is to produce living phenomena for the ring-opening polymerization of phosphoester monomers and polyphosphoesters with narrow molecular weight distribution.[21,22] The solubility of polyphosphoesters in aqueous media can also be controlled by changing both the molecular weight and the structure of the side chain. The newly designed baroplastics swell and form hydrogel states in aqueous media. Moreover, polymer films containing proteinase K were prepared using a low-temperature processing route, after which the enzymatic function of proteinase K was preserved. These water-swollen baroplastics are very suitable for conjugation with biofunctional molecules.

■ EXPERIMENTAL SECTION

Materials. 2-Isopropoxy-2-oxo-1,3,2-dioxaphospholane (IPP) was synthesized as previously reported,[19] purified by vacuum distillation, and stored under argon at 4 °C until use. L-Lactide (LLA; Tokyo Kasei, Tokyo, Japan) was purified by recrystallization from ethyl acetate. Stannous octanoate was purchased from Sigma-Aldrich, Saint Louis, U.S.A. Other chemicals were obtained as extra-purified grade and used without further purification. Distilled water was obtained by purification using a Millipore Milli-Q system, which involves reverse osmosis, ion exchange, and filtration (18.2 MΩ).

Synthesis of Block Copolymers. Polymerization of IPP was performed using a previously described method[21] and poly(IPP) (PIPP) was purified by dialysis in methanol. The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were determined by gel permeation chromatography (GPC) through a Polymer Laboratories MIXED-C column, using a calibration curve based on linear polystyrene standards. For this measurement, chloroform was used as the GPC solvent. In the current study, $PIPP_{78}$ with a number-average degree of polymerization (DP_n) of 78 was used.

The required amounts of LLA, $PIPP_{78}$ (20 g), and stannous octanoate (0.5 mol % of LLA) were placed into a thoroughly dried 50 mL three-necked round-bottomed flask equipped with a magnetic stirrer. After drying in vacuo for 2 h, the polymerization was carried out under reduced pressure at 130 °C for 12 h. The polymerization mixture was dissolved in 20 mL of dichloromethane, and $PIPP_{78}$-*b*-$PLLA_x$ (x: DP_n of PLLA block) was purified by reprecipitation from excess methanol. The formation of $PIPP_{78}$-*b*-$PLLA_x$ was confirmed on the basis of its ^{1}H NMR (α-500, JEOL, Tokyo, Japan) and FT-IR spectra (FT-500, Jasco, Tokyo, Japan). The chemical structure of the synthesized $PIPP_{78}$-*b*-$PLLA_x$ is shown in Figure 1 and Table 1.

Preparation of Polymer Films. Each block copolymer was processed by compression molding under a hydraulic pressure of 35 MPa for 5 min using an Imoto hydraulic press (180C, Imoto, Kyoto, Japan). The processing temperature for $PIPP_{78}$-*b*-$PLLA_x$ was 35 °C, and the PLLA homopolymer was pressed at 130 °C for 5 min.

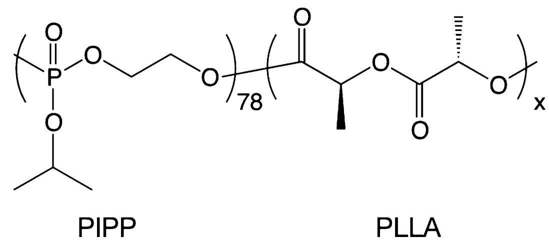

Figure 1. Chemical structure of $PIPP_{78}$-*b*-$PLLA_x$.

Dried polymer films [10 mm (diameter) × 0.5 mm (thick)] were placed in a nylon mesh bag. The bag was soaked in a glass vial containing 100 mL of distilled water at 4 and 37 °C. The weight of the bag was measured after given periods, and the swelling ratio of the film was calculated using

$$\text{swelling degree}(\%) = [(W_s - W_d)/W_s] \times 100 \quad (1)$$

where W_d and W_s are the weights of the dried and swollen films, respectively. The swelling degrees of the polymer films are summarized in Table 2.

Evaluation of Thermal Properties of Block Copolymers. The thermal analysis of $PIPP_{78}$-*b*-$PLLA_x$ was carried out using a differential scanning calorimeter (DSC; Model DSC8230HP, Rigaku, Tokyo, Japan). The temperature was initially raised from room temperature to 100 °C at a heating rate of 5 °C/min. After becoming constant, the temperature was decreased to −100 °C at a cooling rate of 5 °C/min. After holding at −100 °C for 5 min, the temperature was raised to 200 °C at the same heating rate. Then, the temperature was again decreased to 60 °C at the same cooling rate. The glass transition temperature (T_g), cold crystallization temperature (T_{cc}), and melting point (T_m) of the polymer crystal were obtained from the heating DSC curve. On the other hand, the crystallization temperature (T_c) was recorded from the exothermic peak observed in the cooling DSC curve.

Evaluation of Mechanical Properties of Block Copolymers. Tensile tests of the block copolymer films were performed with a tensile testing machine (Autograph AGS-J, Shimadzu Co, Japan). The samples were cut into dog-bone shapes (12.5 mm × 2.5 mm), the crosshead speed was 2 mm/min, and four specimens were tested.

Crystal Structure Analysis. Small-angle X-ray scattering (SAXS) measurements were performed on a Rigaku Nano-Viewer (Rigaku) comprising a Cu Kα radiation source, a three-pinhole-collimated beam, and a Pilatus 100 K 2D detector (Dectris, Baden, Switzerland). The camera length and exposure time were 1253 mm and 10 min, respectively. A pressure cell with diamond windows (Syn Corporation, Kyoto, Japan) was used to measure the effects of pressure in situ on a block copolymer using dry nitrogen as a pressurizing medium at 35 °C. Scattering data were collected for 10 min at each pressure during both upward and downward pressure sweeps.

X-ray diffraction was performed on a Bruker D2 Phaser, using a step-scanning method with Cu Kα radiation at 30 kV and 10 mA, a count rate of 0.5 s per step, and in a 2θ range of 5 to 35°.

The surface morphologies of the microstructures were observed with a scanning probe microscope (SPM; SPM-9700, Shimadzu, Kyoto, Japan) in phase mode.

Preparation and Function of Protein-Embedded Block Copolymer Films. Block copolymer (120 mg) and proteinase K (4.5 mg) were thoroughly mixed in the solid phase, and polymer films were processed under a hydraulic pressure of 35 MPa for 5 min at 35 or 130 °C. The polymer films were cut into disk-shaped specimens 10 mm in diameter. The nylon mesh bags containing the dried polymer films were soaked in 0.01 M Tris buffer solution (pH 9.0) containing 0.05% w/v NaN_3 at 37 °C. The weight loss (%) of the films was calculated by weighing the dried films after soaking them in the buffer for a given period of time. Data are represented as the mean ± the standard deviation (SD). Statistical comparisons were performed with Student's t test.

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Table 1. Characterization of Synthetic Polymers

polymers	IPP/LLA[a] (DP) in feed	IPP/LLA[a] (DP) in copolymer	LLA (wt %)	$M_n \times 10^{-4}$ [b]	M_w/M_n [b]	yield (%)
$PIPP_{78}$-*b*-$PLLA_{77}$	78:75	78:77	46.1	1.18	1.58	58.4
$PIPP_{78}$-*b*-$PLLA_{103}$	78:100	78:103	53.4	1.31	1.51	54.9
$PIPP_{78}$-*b*-$PLLA_{140}$	78:140	78:140	60.9	2.03	1.56	70.1
$PLLA_{103}$		0:103	100	1.48	1.35	48.2

[a]Degree of polymerization (DP) of LLA was determined by ^{1}H NMR. [b]Molecular weight and polydispersity index were determined by gel-permeation chromatography.

Table 2. Swelling Degree of Processed Polymer Films

polymers	4 °C	37 °C
$PIPP_{78}$-*b*-$PLLA_{77}$	33.0 ± 4.2	13.9 ± 1.0
$PIPP_{78}$-*b*-$PLLA_{103}$	17.4 ± 1.1	12.4 ± 0.7
$PIPP_{78}$-*b*-$PLLA_{140}$	16.2 ± 1.7	14.2 ± 0.7
$PLLA_{103}$	2.8 ± 1.1	4.1 ± 0.0

■ RESULTS AND DISCUSSION

Synthesis of Block Copolymers. $PIPP_{78}$ was synthesized using a previously described method.[21] The glass transition temperature of $PIPP_{78}$ was −41.5 °C (Table 3), and $PIPP_{78}$ was obtained in a highly viscous liquid form at room temperature. By using $PIPP_{78}$ as a macroinitiator, $PIPP_{78}$-*b*-$PLLA_x$ was synthesized by ring-opening polymerization of LLA under solvent-free conditions. The DP_n of PLLA could be controlled by the [LLA]/[PIPP] ratio. ^{1}H NMR spectra of $PIPP_{78}$-*b*-$PLLA_x$ are shown in Supporting Information, Figure S1. The molecular weight distribution (M_w/M_n) of the block copolymers was 1.5−1.6. The swelling degrees of the polymer films are summarized in Table 2.

Each polymeric block synthesized was not water-soluble, but swelled in aqueous media because soft segments of the block copolymers, $PIPP_{78}$, were hydrophilic. The swelling degree of the block copolymers decreased with an increase in the DP_n of PLLA. Interestingly, a temperature dependence in the swelling degree of the copolymers was also observed as shown in Table 2. In previous literature, we reported that polyphosphoesters show thermoresponsivity in aqueous media.[21,23] Supporting Information, Figure S2, shows the repeated temperature dependence of the swelling degree of $PIPP_{78}$-*b*-$PLLA_{77}$. The temperature-dependent swelling was reversible with temperature regardless of the number of repetitions. The aqueous solution containing PIPP forms low critical solution temperature (LCST)-type coacervates. Namely, partial dehydration of PIPP occurs as the temperature increases. The phenomenon is strongly related to the structure and molecular weight of the polymers, as well as to the solvent conditions. The thermoresponsive hydration behavior of $PIPP_{78}$-*b*-$PLLA_x$ films is then due to the $PIPP_{78}$ block; this unique property has not been observed in other baroplastics.

Properties of Block Copolymers. Table 3 summarizes the thermal properties of $PIPP_{78}$-*b*-$PLLA_x$. The T_g of $PIPP_{78}$ homopolymers is around −41.5 °C, which is much lower than the T_g of the soft segments of the block copolymers. The hard segments of the block copolymers may influence the T_g of the soft segments because the T_g of the soft segments of the block copolymers slightly increased with an increase in the DP_n of PLLA. The typical DSC heating curve for a block copolymer is shown in Supporting Information, Figure S3. T_g of the hard segments was not observed for block copolymers with $PLLL_{77}$ and $PLLA_{103}$. Supporting Information, Figure S4(A), shows heating DSC curves for $PLLA_{103}$ and the block copolymers. Although exothermic and endothermic peaks due to the cold crystallization and crystal melting of $PLLA_{103}$ during the DSC heating run were observed, the heating DSC curves of each block copolymer only have endothermic peaks. It has been reported that the T_m of PLLA ranges from approximately 130 to 180 °C and increases with increasing molecular weight.[6] Comparing the T_m of $PLLA_{103}$ and $PIPP_{78}$-*b*-$PLLA_{103}$, the T_m of $PIPP_{78}$-*b*-$PLLA_{103}$ was much lower than that of $PLLA_{103}$. The $PIPP_{78}$ block would suppress crystallization of the PLLA block. The crystallinity (X_c) of the hard segments of the block copolymers was calculated using

$$X_c = [(\Delta H_{cc} + \Delta H_m)/\Delta H_{m,100}] \times 100 \quad (2)$$

where ΔH_{cc} (only for $PLLA_{103}$) and ΔH_m are the enthalpies of crystallization and fusion corresponding to the component, respectively, and $\Delta H_{m,100\%}$ is the theoretical enthalpy of the perfect crystal fusion per gram of PLLA (135 J·g^{-1}).[25] The X_c of the block copolymers increased with increasing DP_n of the PLLA.

For every block copolymer, the exothermic peak due to crystallization was observed in the cooling DSC curves, as shown in Supporting Information, Figure S4(B). As for the heating DCS curves, T_c and isothermic enthalpy (ΔH_c) increased with increasing DP_n of the PLLA.

Table 3. Thermal Properties and Crystallinity of Synthetic Polymers

polymers	T_g[a] (°C) PIPP	T_g[a] (°C) PLLA	T_g'[b] (°C)	T_{cc}[a] (°C)	ΔH_{cc}[a] (J·g^{-1})	T_m[a] (°C)	H_m[a] (J·g^{-1})	T_c[c] (°C)	H_c[c] (J·g^{-1})	X_c[c] (%)	X_c[d] (%)
$PIPP_{78}$-*b*-$PLLA_{77}$	−28.3	N.D.	−0.8	N.D.	N.D.	161.8	44.8	98.7	28.3	33.2	14.5
$PIPP_{78}$-*b*-$PLLA_{103}$	−27.8	N.D.	6.9	N.D.	N.D.	160.3	46.2	99.7	33.7	34.2	23.3
$PIPP_{78}$-*b*-$PLLA_{140}$	−27.4	58.5	15.2	N.D.	N.D.	168.4	59.0	104.7	42.0	43.7	38.9
$PLLA_{103}$		67.1		105.8	30.7	173.4	82.2	103.2	71.0	83.6	86.8
$PIPP_{78}$	−41.5										

[a]Determined by DSC at a heating rate of 5 °C/min. [b]T_g' is theoretical T_g values of block copolymers under pressure (miscible state). T_g' values were calculated according to the Fox equation.[24] [c]Determined by DSC at a cooling rate of 5 °C/min. [d]Determined by XRD.

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The stress−strain curves and mechanical properties for the block copolymers are shown in Table 4 and Supporting Information, Figure S5, respectively. The Young's modulus and tensile strength of the block copolymers increased with increasing DP_n of the PLLA. In contrast, elongation at the breaking point of the block copolymer specimens varied in the opposite manner. Although $PLLA_{103}$ film is brittle due to high crystallinity, the elastic properties of the polymer films could be varied by copolymerization with amorphous PIPP.

Table 4. Mechanical Properties of Synthetic Polymers[a]

polymers	Young's modulus (MPa)	elongation (%)	stress at break (MPa)
$PIPP_{78}$-*b*-$PLLA_{77}$	8.3 ± 1.0	82.1 ± 4.4	0.2 ± 0.0
$PIPP_{78}$-*b*-$PLLA_{103}$	98.8 ± 7.8	40.2 ± 9.0	2.4 ± 0.4
$PIPP_{78}$-*b*-$PLLA_{140}$	371.1 ± 31.4	6.7 ± 1.7	8.4 ± 0.5
$PLLA_{103}$	1989.3 ± 125.2	5.0 ± 3.8	10.2 ± 6.1

[a]"±" denotes standard deviation (n = 4).

Figure 2 shows the SAXS profiles of $PIPP_{78}$-*b*-$PLLA_{140}$ under ambient and 50 MPa pressures. At ambient pressure, a peak is observed at 4.24×10^{-2} Å^{-1}. This peak can be attributed to the formation of a periodic structure, such as a lamellar structure, upon microphase separation of PIPP and PLLA. The average structural length Λ_{avg} was 14.8 nm, as determined by

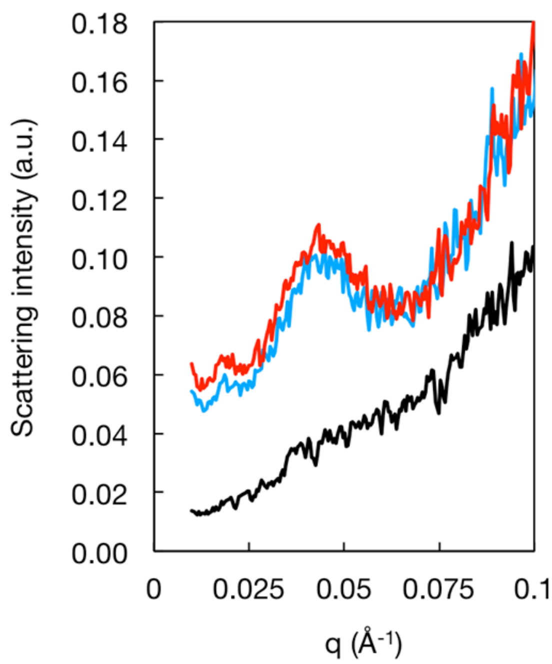

Figure 2. SAXS patterns for $PIPP_{78}$-*b*-$PLLA_{140}$ under atmospheric and hydraulic pressure at 35 °C: blue line, atmospheric pressure; black line, 50 MPa hydraulic pressure; red line, atmospheric pressure (after removing hydraulic pressure).

$$\Lambda_{avg} = \frac{2\pi}{q_{max}} \quad (3)$$

The calculated size agrees well with the lamellar structure observed by SPM, as shown in Supporting Information, Figure S6. The scattering peak was completely diminished under 50 MPa of applied pressure. This result indicates that the PLLA phase is dissolved in the fluidized soft $PIPP_{78}$ phase. Moreover, the peak was completely recovered after removing the pressure. The pressure-induced phase transition between phase-separated and miscible states is reversible. From this SAXS measurement, it was clarified that $PIPP_{78}$-*b*-$PLLA_{140}$ showed pressure-induced miscibility. Similar behavior was observed for various non-degradable block copolymers of polystyrene and other poly(*n*-alkyl methacrylate).[11−14,26] Generally, a two-component system shows an upper disorder-to-order transition (UDOT) upon heating. In such a system, the polymer chains comprising two blocks undergo an order-to-disorder transition upon pressurization. According to the decrement of the phase-transition temperature under pressure, the two components could be miscible, and the PLLA crystals of the hard segment disappeared.

Figure 3 shows a photograph of the low-temperature processability of $PIPP_{78}$-*b*-$PLLA_{140}$. A given amount of raw polymer was pressed into a human-shaped mold under a hydraulic pressure of 35 MPa for 5 min at ambient temperature, and a transparent molded film was obtained. The theoretical T_g values (T_g') of block copolymers at miscible state (under pressure) is shown in Table 3, which are much lower than the molded temperature.

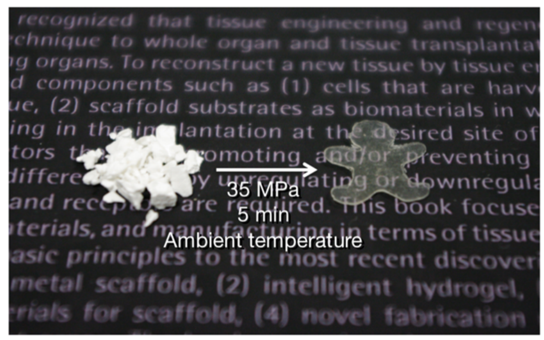

Figure 3. Photograph of the low-temperature processing of $PIPP_{78}$-*b*-$PLLA_{140}$ under 35 MPa for 5 min at 35 °C.

The X_c of block copolymers was also studied using XRD. The scattering profiles are shown in Figure 4. For all of the block copolymers, crystalline peaks typical of PLLA were observed at 16.7 [(200) and (110) Miller planes], 19.1 [(203) and (113)], and 22.3° [(210)].[15] The peak observed between

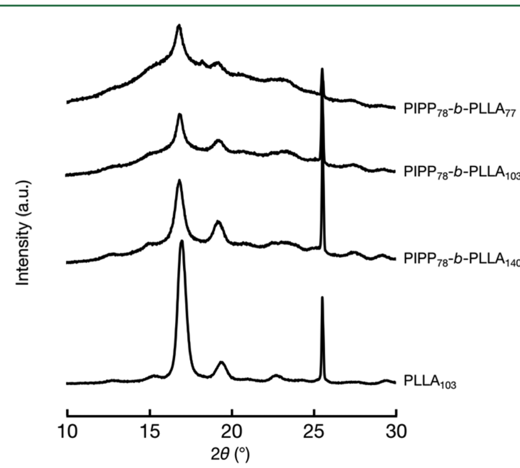

Figure 4. XRD patterns for PLLA and $PIPP_{78}$-*b*-$PLLA_x$.

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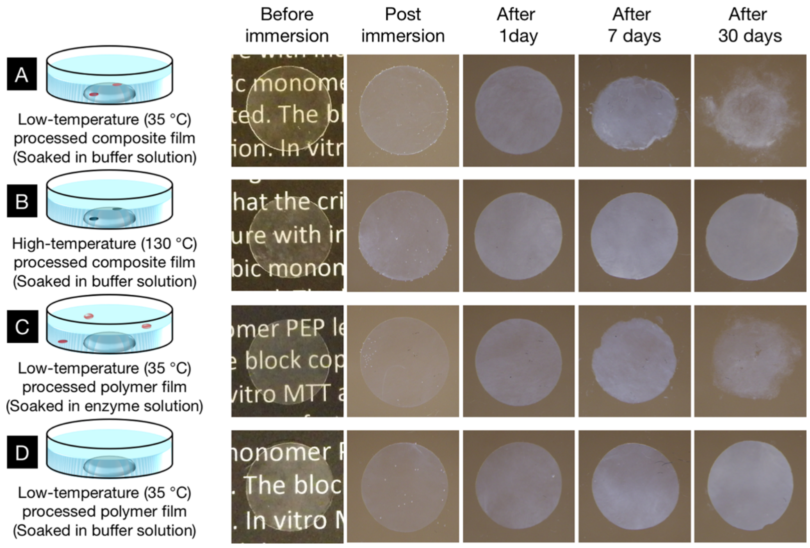

Figure 5. Degradation of polymer films by hydrolysis. (A) Low-temperature (35 °C) processed PIPP$_{78}$-*b*-PLLA$_{140}$ composite film with proteinase K. The film was soaked in Tris buffer solution. (B) High-temperature (130 °C) processed PIPP$_{78}$-*b*-PLLA$_{140}$ composite film with proteinase K. The film was soaked in Tris buffer solution. (C) Low-temperature processed PIPP$_{78}$-*b*-PLLA$_{140}$ film. The film was soaked in Tris buffer solution containing proteinase K. (D) Low-temperature processed PIPP$_{78}$-*b*-PLLA$_{140}$ film. The film was soaked in Tris buffer solution.

25° and 26° is a measurement artifact. The amorphous halo became larger relative to the peaks as the DP$_n$ of PLLA decreased. The crystallinity calculated from the XRD data is also summarized in Table 3. This result is similar to the X_c determined by DSC analysis. The effect of multiple processing steps on X_c was also investigated, as shown in Supporting Information, Figure S7. The processing time did not show any adverse effect on the XRD profile of PIPP$_{78}$-*b*-PLLA$_{140}$, and X_c did not change after remolding. The reprocessability of the block copolymers could then be confirmed.

Function of Enzyme Incorporated into Block Copolymer Films. Proteinase K is a fungal protease produced by the mold *Tritirachium album*.[27] The enzyme has a molecular weight estimated by gel filtration of 18500 ± 500, an isoelectric point of 8.9, and an optimum activity pH range of 7.5−12.0.[28] Reeve and co-workers studied enzyme-catalyzed degradation of poly(lactic acid) (PLA) using proteinase K. Proteinase K preferentially degraded PLLA compared with D-form PLA, and the degradation favorably occurred in the amorphous domains.[29]

Figure 5 shows the function of proteinase K embedded in PIPP$_{78}$-*b*-PLLA$_{140}$ films. Despite the addition of proteinase K, transparent films were obtained. When the films were soaked in buffer solution, the films became turbid due to partial hydration of polyphosphoesters. The polymer film was soaked in buffer solution without having any contact with proteinase K, and the shape of the film did not change. In contrast, degradation of the low-temperature processed polymer film containing proteinase K was observed after 7 days of soaking in buffer solution, and the film was significantly eroded after 30 days of soaking in buffer solution. A similar tendency was observed for the polymer film after soaking in buffer solution containing the same amount of proteinase K. When the enzyme-containing polymer film was processed at 130 °C, erosion of the film was not observed. Proteinase K incorporated in the film lost enzymatic activity after heat treatment. The weight loss of the polymer films soaked in buffer solution is shown in Figure 6. The weight of polymer films soaked in enzyme-containing buffer and low-temperature processed enzyme-containing

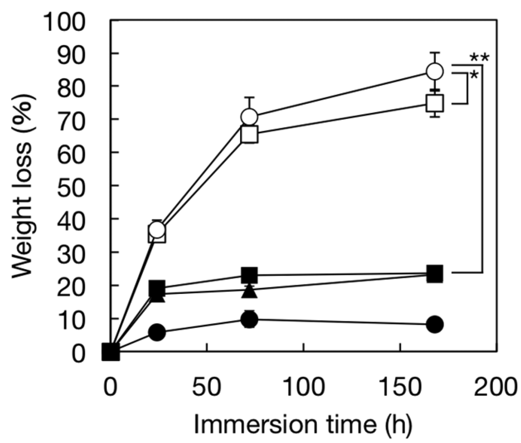

Figure 6. Weight loss of polymer films by hydrolysis. (□) Low-temperature (35 °C) processed PIPP$_{78}$-*b*-PLLA$_{140}$ composite film with proteinase K. The film was soaked in Tris buffer solution. (■) High-temperature (130 °C) processed PIPP$_{78}$-*b*-PLLA$_{140}$ composite film with proteinase K. The film was soaked in Tris buffer solution. (○) Low-temperature processed PIPP$_{78}$-*b*-PLLA$_{140}$ film. The film was soaked in Tris buffer solution containing proteinase K. (▲) Low-temperature processed PIPP$_{78}$-*b*-PLLA$_{140}$ film. The film was soaked in Tris buffer solution. (●) High-temperature (130 °C) processed PLLA$_{103}$ film. The film was soaked in Tris buffer solution containing proteinase K. *p > 0.01 vs □; **p < 0.01 vs ■.

polymer films drastically decreased as a function of increasing soaking period. In contrast, enzyme-containing polymer films processed at 130 °C exhibited only a 20% decrease in weight after the first 24 h. This phenomenon is in good agreement with the weight change of polymer films soaked in buffer solution without proteinase K. Therefore, the slight weight loss is not due to enzymatic function. When the PLLA$_{103}$ membrane was soaked in buffer solution containing proteinase K, the weight loss was significantly less for the duration of testing. The hydration of block copolymer membrane is also a key factor in the hydrolytic degradation of films, and PIPP is suitable for achieving this condition.

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■ CONCLUSIONS

In the current study, we first present the advantages of baroplastics for use in composite polymer materials with biomolecules. The block copolymers comprising aliphatic polyester and polyphosphoester segments showed good pressure-induced miscibility, and low-temperature processing of the block copolymers could be achieved. The mechanical properties of the block copolymers can be varied by changing the amounts of PLLA and PIPP. The function of proteinase K incorporated into the block copolymers was preserved after processing under hydraulic pressure at ambient temperature. The partial hydration behavior of the block copolymers also creates suitable conditions for biomolecule activity. We anticipate that various composite materials comprising PIPP-*b*-PLLA and proteins will be obtained by a simple mixing technique, and that they will be applied in industrial and biomedical fields.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.6b00641.

^{1}H NMR spectra, DSC thermograms, and stress−strain curves of polymers, the repeated temperature dependence of the swelling degree of $PIPP_{78}$-*b*-$PLLA_{77}$ film, topological SPM image of the $PIPP_{78}$-*b*-$PLLA_{140}$ film surface, and XRD patterns for $PIPP_{78}$-*b*-$PLLA_{140}$ film after remolding multiple times (PDF).

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Notes

The authors declare no competing financial interest.

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