Synthesis and Characterization of Poly[(*R*,*S*)-3-hydroxybutyrate-*b*-6-hydroxyhexanoate] as a Compatibilizer for a Biodegradable Blend of Poly[(*R*)-3-hydroxybutyrate] and Poly(6-hydroxyhexanoate)

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ABSTRACT: A block copolymer (P[*R*,*S*]-3HB-*b*-6HH]) of atactic poly[(*R*,*S*)-3-hydroxybutyrate] (P[(*R*,*S*)-3HB]) and poly(6-hydroxyhexanoate) (P(6HH)) was prepared by ring-opening polymerization of (*R*,*S*)-β-butyrolactone followed by the subsequent polymerization of ε-caprolactone in the presence of $Zn(C_2H_5)_2/H_2O$ catalyst. The block copolymer was an A–B diblock copolymer comprising atactic P[(*R*,*S*)-3HB] (A) and P(6HH) (B) segments. The block copolymer was used as a compatibilizer for the immiscible blend of microbial poly[(*R*)-3-hydroxybutyrate] (P[(*R*)-3HB]) and P(6HH). The addition of a small amount of the block copolymer into the P[(*R*)-3HB]/P(6HH) blend reduced the size of the P(6HH) dispersed domains. Good adhesion of the interface between P[(*R*)-3HB] and P(6HH) was achieved by the intervention of the block copolymer, resulting in an improvement of mechanical properties of the blends. The enzymatic degradation of blend films was carried out at 37 °C and pH 7.4 in a 0.1 M phosphate solution of an extracellular PHB depolymerase from *Alcaligenes faecalis* T1. The enzymatic degradation took place on the surface of the blend films, and the erosion rate decreased in the following order: P[(*R*)-3HB]/P(6HH) [75/25 (wt %)] > P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] [71/24/5 (wt %)] > P[(*R*)-3HB] ≫ P(6HH).

Introduction

An optically active poly[(*R*)-3-hydroxybutyrate] (P[(*R*)-3HB]) of pure isotactic structure is synthesized by a variety of bacteria as an intracellular storage material of carbon and energy.[1] Microbial P[(*R*)-3HB] is a biodegradable and biocompatible thermoplastic with a melting temperature around 180 °C and has attracted industrial attention as an environmentally degradable material for a wide range of agricultural, marine, and medical applications.[2–4] The mechanical properties of Young's modulus (3.5 GPa) and the tensile strength (40 MPa) of the P[(*R*)-3HB] film are close to those of isotactic polypropylene. The extension (6%) needed to break P[(*R*)-3HB] is, however, markedly lower than that (400%) of polypropylene. The stiffness and brittleness of P[(*R*)-3HB] due to high crystallinity have limited its practical use.[5]

Two approaches have been extensively studied to improve the physical properties of P[(*R*)-3HB]. One approach is the microbial synthesis of copolymers containing hydroxyalkanoate monomeric units other than the (*R*)-3HB unit. For example, random copolyesters of (*R*)-3HB with (*R*)-3-hydroxyvalerate[3,6] or 4-hydroxybutyrate[7,8] have been produced from alkanoic acids by *Alcaligenes eutrophous*. The physical and thermal properties of microbial copolyesters can be regulated by varying their molecular structure and copolymer compositions.[9–12]

The second approach is blending P[(*R*)-3HB] with biodegradable polymers such as poly(ethylene oxide),[13–15] poly(vinyl alcohol),[16] polysaccharides,[17] poly(6-hydroxyhexanoate),[18,19] poly(3-hydroxypropionate),[20] poly(ethylene adipate),[20] atactic poly(3-hydroxybutyrate),[21–23] and microbial poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate).[20,24] For example, it has been found that atactic poly[(*R*,*S*)-3-hydroxybutyrate] (P[(*R*,*S*)-3HB]) is miscible with isotactic P[(*R*)-3HB] in the amorphous region[23] and that the miscible blend is degraded in the presence of an extracellular PHB depolymerase from *Alcaligenes faecalis*.[20] In contrast, poly(6-hydroxyhexanoate) (P(6HH)) is not degraded by PHB depolymerase but eroded by microbial lipases.[25] Biodegradable P(6HH) of high molecular weights is, however, immiscible with P[(*R*)-3HB] in the amorphous region, and the immiscible blend has the emulsion structure of a macrophase separation.[19]

Several block copolymers have been used as compatibilizers of immiscible polymer blends to improve the mechanical properties.[26–30] Recently, Reeves *et al.*[31] have prepared a diblock copolymer of isotactic P[(*R*)-3HB] and P(6HH) by the polymerization of ε-caprolactone with a P[(*R*)-3HB]—O— $Al(C_2H_5)_2$ macroinitiator which was obtained by the reaction of P[(*R*)-3HB] with $Al(C_2H_5)_3$. The diblock copolymer was not used as a compatibilizer of the P[(*R*)-3HB]/P(6HH) blend, because the molecular weight of the block copolymer was low.

In this paper, we synthesized a diblock copolymer (P[(*R*,*S*)-3HB-*b*-6HH]) of atactic P[(*R*,*S*)-3HB] and P(6HH) of high molecular weight in the presence of $Zn(C_2H_5)_2/H_2O$ catalyst and characterized the block copolymer. The block copolymer was used as a compatibilizer for the immiscible blend of microbial P[(*R*)-3HB] and P(6HH) to improve the mechanical properties. The enzymatic degradation of blend films was studied in the aqueous solution of an extracellular PHB depolymerase purified from *A. faecalis*.[32]

Experimental Section

Materials. The microbial poly[(*R*)-3-hydroxybutyrate] (P[(*R*)-3HB]) ($\bar{M}_n$ = 650 000, $\bar{M}_w/\bar{M}_n$ = 1.8) was produced by *A. eutrophous* from fructose[33] and purified by precipitation in hexane from chloroform solution at room temperature. (*R*,*S*)-

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Table 1. Molecular Weights and Polydispersities of P[(R,S)-3HB-b-6HH] Block Copolymers and P[(R,S)-3HB] Prepolymers

	P[(R,S)-3HB][a]			P[(R,S)-3HB-b-6HH][b]			
		molecular weight[c]			molecular weight[c]		
sample no.	β-BL/Zn, mol/mol	$10^{-3}\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	ε-CL/Zn, mol/mol	$10^{-3}\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	polymer yield, %
1	50	19.7	1.15	50	42.8	1.43	98
2	50	21.0	1.18	100	69.5	1.50	97
3	100	44.8	1.22	100	81.2	1.54	98

[a] Polymerization conditions of (R,S)-β-BL: $Zn(C_2H_5)_2H_2O$ (1/0.6) = 0.09 g (R,S)-β-BL/solvent (1,2-dichloroethane) = 0.05 (v/v), reaction temperature = 60 °C, reaction time = 5 days. [b] Polymerization conditions of ε-CL: reaction temperature = 40 °C, reaction time = 5 days. [c] Determined by GPC using polystyrene standards.

β-Butyrolactone ((R,S)-β-BL) and ε-caprolactone (ε-CL) were dried on CaH_2 and distilled under nitrogen. Poly(6-hydroxyhexanoate) (P(6HH)) was prepared by ring-opening polymerization of ε-CL in the presence of poly(methylaluminoxane) (MAO) catalyst. The polymerization of ε-CL with MAO was carried out in toluene at 60 °C for 7 days. The number-average molecular weight and polydispersity of P(6HH) were $\bar{M}_n$ = 59 000 and $\bar{M}_w/\bar{M}_n$ = 1.5, respectively.

Catalyst Preparation. The $Zn(C_2H_5)_2/H_2O$ (1/0.6) catalyst was prepared by a reported method.[34] $Zn(C_2H_5)_2$ was reacted with deoxygenated water at a molar ratio of 1/0.6 ($Zn(C_2H_5)_2/H_2O$) in dry 1,4-dioxane (distilled over Na), followed by freeze-drying of the reaction mixture. A yellow powder was obtained.

Synthesis of P[(R,S)-3HB-b-6HH]. The atactic P[(R,S)-3HB] was prepared by ring-opening polymerization of (R,S)-β-BL in the presence of $Zn(C_2H_5)_2/H_2O$ catalyst. The polymerization of (R,S)-β-BL with $Zn(C_2H_5)_2/H_2O$ catalyst was carried out in 1,2-dichloroethane at 60 °C for 5 days. Then, ε-CL was added to the solution of P[(R,S)-3HB]. The block copolymerization with ε-CL was carried out at 40 °C for 5 days and terminated by adding methanol. The copolymer was dissolved in chloroform and precipitated in methanol. The precipitate was dried *in vacuo* at room temperature.

Preparation of Blends. Films of blends were prepared by conventional solvent-casting techniques from chloroform solution using glass Petri dishes as casting surfaces. The films were then aged at least 3 weeks at room temperature to reach equilibrium crystallinity prior to analysis.[35]

Enzymatic Degradation. Extracellular PHB depolymerase was purified to electrophoretic homogeneity from *A. faecalis* T1[32] and used in this study. Enzymatic degradations of polymer blend films were carried out at 37 °C in a 0.1 M phosphate buffer (pH 7.4) for 12 h. The blend films (initial weight, 5 mg; initial film dimension, 10 × 10 mm in size and 0.05 mm thick) were placed in small bottles containing 1.0 mL of the buffer. The reaction was started by the addition of 40 μL of an aqueous solution of PHB depolymerase (8 μg). The reaction solution was incubated at 37.0 ± 0.1 °C with shaking. After the reaction, sample films were washed with distilled water and dried to constant weight *in vacuo*.

Analytical Procedures. All molecular weight data were obtained by gel permeation chromatography at 40 °C, using a Shimadzu 6A GPC system and a 6A refractive index detector with Shodex K-80M and K-802 columns. Chloroform was used as the eluent at a flow rate of 0.8 mL/min, and sample concentrations of 1.0 mg/mL were applied. Polystyrene standards with a low polydispersity were used to make a calibration curve.

The ^{13}C NMR spectra at 125 MHz of polymers were recorded on a JEOL GX-500 spectrometer at 27 °C in a $CDCl_3$ solution of polymer (30 mg/mL) with a 5.5 μs pulse width (45° pulse angle), 5 s pulse repetition, 25 000 Hz spectral width, 64K data points, and 13 000 accumulations.

Differential scanning calorimetry (DSC) data of polymers were recorded in the temperature range −150 to +200 °C on a Shimadzu DSC-50 equipped with a cooling accessory under a nitrogen flow of 30 mL/min. Samples of 10 mg were encapsulated in aluminum pans and heated from 0 to 200 °C at a rate of 10 °C/min. The melting temperature (T_m) and enthalpy of fusion (ΔH_m) were determined from the DSC endotherms. For measurement of the glass-transition temperature (T_g), the samples were maintained at 200 °C for 1 min and then rapidly quenched at −150 °C. They were heated from −150 to +200 °C at a heating rate of 20 °C/min. The T_g was taken as the midpoint of the heating capacity change.

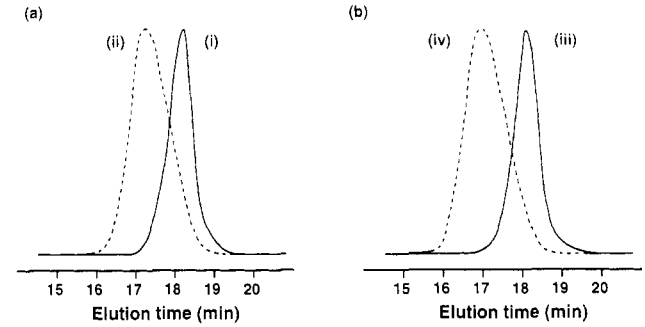

Figure 1. The GPC curves of P[(R,S)-3HB] prepolymers and P[(R,S)-3HB-b-6HH] block copolymers: (a) [sample 1] (i) P[(R,S)-3HB] ($\bar{M}_n$ = 19 700, $\bar{M}_w/\bar{M}_n$ = 1.15), (ii) P[(R,S)-3HB-b-6HH] ($\bar{M}_n$ = 42 800, $\bar{M}_w/\bar{M}_n$ = 1.43); (b) [sample 2] (iii) P[(R,S)-3HB] ($\bar{M}_n$ = 21 000, $\bar{M}_w/\bar{M}_n$ = 1.18), (iv) P[(R,S)-3HB-b-6HH] ($\bar{M}_n$ = 69 500, $\bar{M}_w/\bar{M}_n$ = 1.50).

The stress–strain curves of films were obtained at 23 °C at a strain rate of 20 mm/min on an Imada tensile machine (Model SV-50). Mechanical tensile data were calculated from such curves on an average of three specimens.

The surface appearances of films were obtained with a scanning electron microscope (JEOL JSM-T220) after gold coating of the films using an ion coater. Morphologies of films were observed with an optical microscope (Nikon OPTIPHOTO-2) equipped with a phase contrast lens.

Results and Discussion

Synthesis of P[(R,S)-3HB-b-6HH] Block Copolymer. The block copolymers of atactic P[(R,S)-3HB] and P(6HH) were prepared as follows. Atactic P[(R,S)-3HB] was first synthesized by the ring-opening polymerization of (R,S)-β-butyrolactone ((R,S)-β-BL) with $Zn(C_2H_5)_2/H_2O$ catalyst in 1,2-dichloroethane at 60 °C for 5 days. Then, ε-caprolactone (ε-CL) was added to the solution of P[(R,S)-3HB], and the sequential block copolymerization with ε-CL was carried out at 40 °C for 5 days. The produced copolymers were characterized by GPC measurement. The results are given in Table 1. The block copolymers were produced in high yields at different mole ratios of ε-CL to β-BL, and they had high molecular weights ($\bar{M}_n$ = 42 800–81 200) and narrow molecular weight distributions ($\bar{M}_w/\bar{M}_n$ = 1.43–1.54). Figure 1 shows typical GPC curves of P[(R,S)-3HB] prepolymers and of the block copolymers. The GPC curves of diblock copolymers are clearly shifted toward high molecular weights relative to those of P[(R,S)-3HB] prepolymers, indicating the formation of a diblock copolymer of P[(R,S)-3HB] and P(6HH) free of homopolymer impurities. Thus, the sequential polymerization of ε-CL with a living P[(R,S)-3HB] resulted in the formation of a diblock copolymer (P[(R,S)-3HB-b-6HH]). As can be seen from Table 1, the polydispersities ($\bar{M}_w/\bar{M}_n$ = 1.43–1.54) of diblock copolymers were larger than those (1.15–1.22) of P[(R,S)-3HB] prepolymers. A slow chain-terminating reaction may take place during the polymerization of ε-CL.

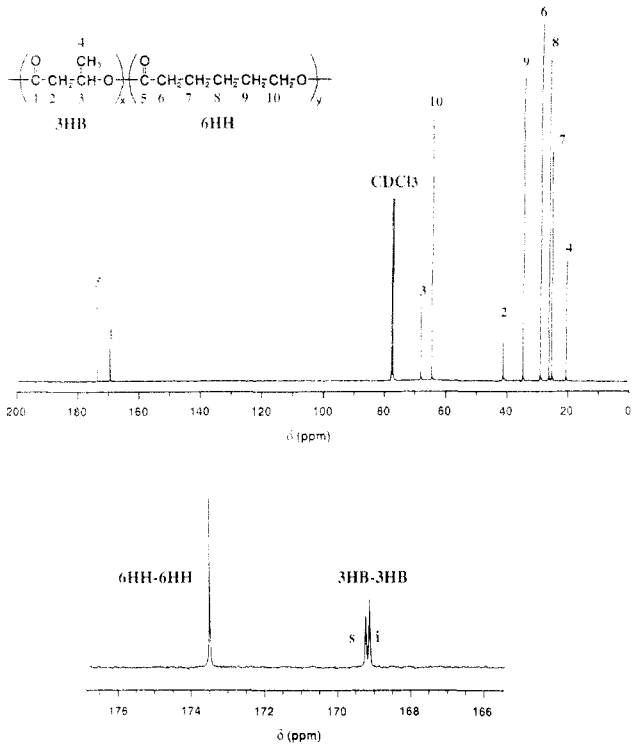

Figure 2. 125 MHz ^{13}C NMR spectrum of the P[(*R*,*S*)-3HB-*b*-6HH] block copolymer (sample 1) and the carbonyl carbon resonance in the ^{13}C NMR spectrum.

The 125 MHz ^{13}C NMR spectrum of a P[(*R*,*S*)-3HB-*b*-6HH] block copolymer (sample 1) is shown in Figure 2, together with the chemical shift assignment of each ^{13}C resonance. The ^{13}C NMR spectrum confirms that the diblock copolymer is composed of atactic P[(*R*,*S*)-3HB] and P(6HH) segments. In the expanded ^{13}C NMR spectrum of carbonyl carbon resonances in Figure 2, no peaks of alternating 3HB–6HH sequences are detected, indicating that the transesterification reaction did not occur during the block copolymerization. The isotactic diad fraction of the P[(*R*,*S*)-3HB] segment was 0.51, as determined from the carbonyl carbon resonance.

The thermal properties of P[(*R*,*S*)-3HB-*b*-6HH] block copolymers are listed in Table 2, together with those of isotactic P[(*R*)-3HB], atactic P[(*R*,*S*)-3HB], and P(6HH) homopolymers. The bacterial P[(*R*)-3HB] was a partially crystalline polymer, and the melting temperature (T_m) and glass-transition temperature (T_g) were 178 and 4 °C, respectively. The atactic P[(*R*,*S*)-3HB] was an amorphous polymer, and the T_g was observed at 3 °C. P(6HH) homopolymer had T_m at 57 °C and T_g at −70 °C. Figure 3 shows a typical DSC curve of the block copolymer (sample 1). Two T_g's at −67 °C for the P(6HH) sequence and 2 °C for the P[(*R*,*S*)-3HB] sequence were observed, together

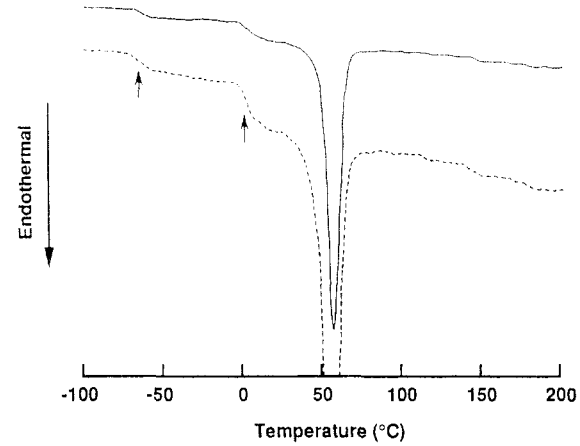

Figure 3. DSC curve of the P[(*R*,*S*)-3HB-*b*-6HH] block copolymer (sample 1).

with one T_m at 60 °C for the P(6HH) sequence. These T_m and T_g values for each sequence were almost the same as the values of each homopolymer. This result suggests that there is a microphase separation as a result of the immiscibility between P[(*R*,*S*)-3HB] and P(6HH) sequences in the amorphous region.

Properties and Morphologies of Blend Films. Table 2 gives the thermal data for the blend films of P[(*R*)-3HB] ($\bar{M}_n$ = 650 000) with P(6HH) ($\bar{M}_n$ = 59 000). The enthalpies of fusion (ΔH_m) are represented as the value per gram of each polymer component in the blend. The T_m values of ternary blends (P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH]) were almost the same as those of the binary blend of P[(*R*)-3HB] with P(6HH). The ΔH_m values of the P[(*R*)-3HB] component in the ternary blends were not influenced by the addition of block copolymer, but the ΔH_m values of the P(6HH) component decreased in the presence of block copolymer. This result suggests that the atactic P[(*R*,*S*)-3HB] sequence of the block copolymer is excluded from the crystalline phase of P[(*R*)-3HB], but that the P(6HH) sequence is included in the crystalline phase of the P(6HH) component.

Figure 4 shows the phase-contrast optical micrographs of the solvent-cast blend films of P[(*R*)-3HB]/P(6HH) (a) and P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] (b). The micrograph of the solution-cast film of P[(*R*)-3HB] (Figure 4c) shows an optical contrast between the crystalline and amorphous phases in the P[(*R*)-3HB] spherulites. In a previous paper,[19] we reported that the immiscible blend of P[(*R*)-3HB]/P(6HH) had the emulsion structure of a macrophase separation as a consequence of the lack of the interfacial adhesion between P[(*R*)-3HB] and P(6HH) components. Figure 4a shows that large P(6HH) dispersed domains of 5–10 μm in diameter are present in the blend of P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)]. The addition

Table 2. Thermal Properties of Films of Homopolymers, Block Copolymers, and Their Blends

sample	blend composition, wt ratio	T_g, °C		P(3HB)		P(6HH)	
		P(3HB)	P(6HH)	T_m, °C	ΔH_m, J/g	T_m, °C	ΔH_m, J/g
P[(*R*)-3HB] ($\bar{M}_n$ = 650 000, $\bar{M}_w/\bar{M}_n$ = 1.8)		4		178	91		
P[(*R*,*S*)-3HB] ($\bar{M}_n$ = 20 000, $\bar{M}_w/\bar{M}_n$ = 1.1)		3			0		
P(6HH) ($\bar{M}_n$ = 59 000, $\bar{M}_w/\bar{M}_n$ = 1.5)			−70			57	68
P[(*R*,*S*)-3HB-*b*-6HH] (sample 1)		2	−67		0	60	63
P[(*R*,*S*)-3HB-*b*-6HH] (sample 2)		4	n.d.[b]		0	61	73
P[(*R*,*S*)-3HB-*b*-6HH] (sample 3)		3	n.d.[b]		0	61	65
P[(*R*)-3HB]/P(6HH)	75/25	4	n.d.[b]	178	96	57	64
P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH][a]	81/14/5	4	n.d.[b]	178	94	57	60
	71/24/5	5	n.d.[b]	178	98	57	53
	68/23/9	3	n.d.[b]	177	96	57	55

[a] Sample 1 in Table 1. [b] Not detected.

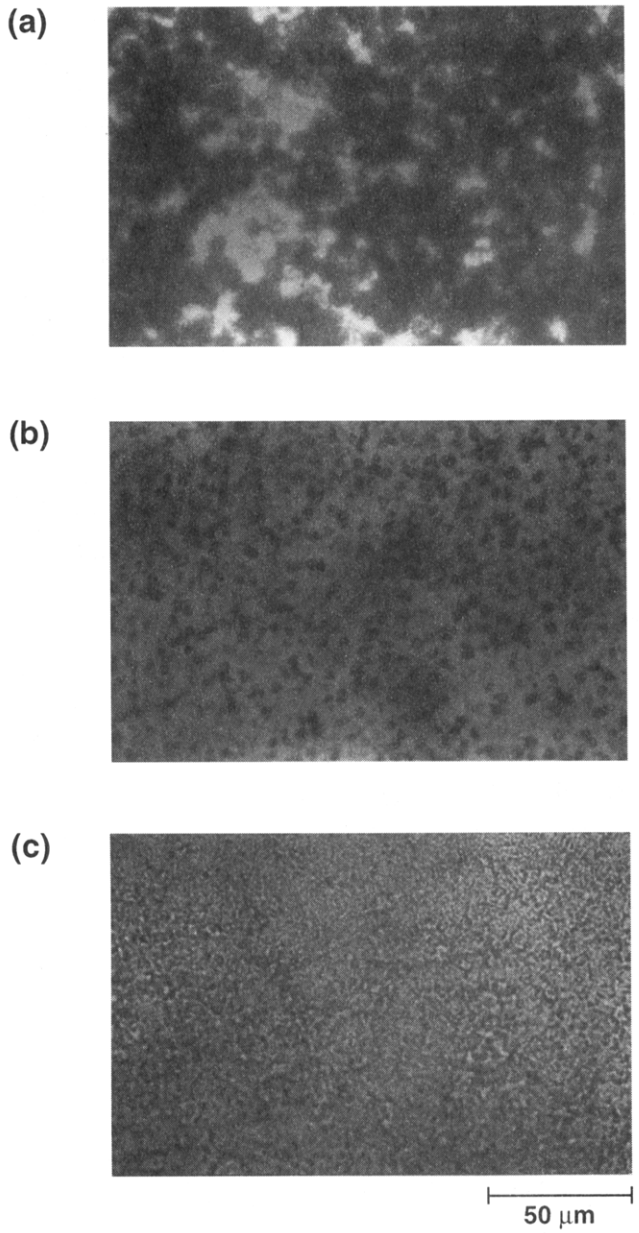

Figure 4. Phase-contrast optical micrographs of the solvent-cast blend films: (a) P[(*R*)-3HB]/P(6HH) = 75/25 (wt ratio); (b) P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] (sample 1) = 71/24/5 (wt ratio); (c) P[(*R*)-3HB] homopolymer.

of a small amount (5 wt %) of block copolymer (sample 1) into the immiscible blend of P[(*R*)-3HB]/P(6HH) [71/24 (wt ratio)] remarkedly reduced the size of P(6HH) dispersed domains to 1–2 μm in diameter (Figure 4b). This result suggests that the block copolymer of atactic P[(*R*,*S*)-3HB] and P(6HH) functions as a compatibilizer for the immiscible blend of isotactic P[(*R*)-3HB] and P(6HH). In previous papers,[20,22] we reported that atactic P[(*R*,*S*)-3HB] was miscible with isotactic P[(*R*)-3HB] in the amorphous region.

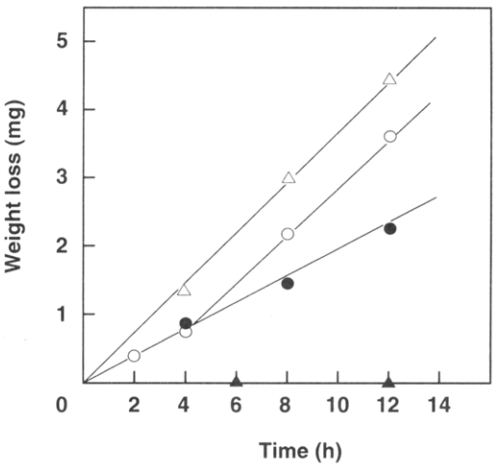

Figure 5. Enzymatic degradation (erosion) profiles of the blend films in the aqueous solution of PHB depolymerase (8 μg) at 37 °C and pH 7.4: (●) P[(*R*)-3HB]; (▲) P(6HH); (△) P[(*R*)-3HB]/P(6HH) = 75/25 (wt ratio); (○) P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] = 71/24/5 (wt ratio).

Comparative experiments on mechanical properties were carried out for the immiscible binary blend of P[(*R*)-3HB]/P(6HH) and the ternary blends containing P[(*R*,*S*)-3HB-*b*-6HH] block copolymers as a compatibilizer. Table 3 gives the mechanical properties of the blend films. The measured value (1.6 GPa) of Young's modulus of the P[(*R*)-3HB] film was about half of the reported value (3.5 GPa). In this study, we used the solvent-cast films as the tensile-testing specimens, while the melt-crystallized samples were used in the previous works.[3,10] De Koning et al.[36] reported that the modulus of P[(*R*)-3HB] samples was affected by both the processing of sample preparation and the aging time. The tensile strength and elongation to break at 23 °C of the P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)] blend film were 22 MPa and 10%, respectively. When small amounts (5–9 wt %) of block copolymers of P[(*R*,*S*)-3HB] and P(6HH) were added into the immiscible blend, the elongation to break of the ternary blends (P[(*R*)-3HB]/P(6HH)/block copolymer) was increased to 68%. Thus, the P[(*R*)-3HB]/P(6HH) blend films became flexible and tough by the addition of P[(*R*,*S*)-3HB-*b*-6HH] block copolymer as a compatibilizer.

Enzymatic Degradation of Blend Films. Enzymatic degradations of P[(*R*)-3HB]/P(6HH) blend films were carried out for 12 h at 37 °C in a 0.1 M phosphate buffer of PHB depolymerase from *A. faecalis* T1. Figure 5 shows the weight loss (erosion) profiles of the films of P[(*R*)-3HB], P(6HH), P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)], and P[(*R*)-3HB]/P(6HH)/P[*R*,*S*)-3HB-*b*-6HH] [71/24/5 (wt ratio)] as a function of enzymatic degradation time. The weight loss of P[(*R*)-3HB] and P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)] increased proportionally with reaction time. As reported in a previous paper,[19] the rate of enzymatic degradation on the surface of the immiscible P[(*R*)-3HB]/P(6HH) blend film was faster than that of the P[(*R*)-3HB] film. In contrast, no weight loss of the P(6HH) film was detected, because PHB depolymerase is

Table 3. Mechanical Properties of Blend Films at 23 °C

sample	blend composition, wt ratio	Young's modulus, MPa	tensile strength, MPa	elongation to break, %
P[(*R*)-3HB]		1560	38	5
P[(*R*)-3HB]/P(6HH)	75/25	740	22	10
P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH][a]	81/14/5	530	22	38
	71/24/5	340	17	62
	63/23/9	320	15	68

[a] Sample 1 in Table 1.

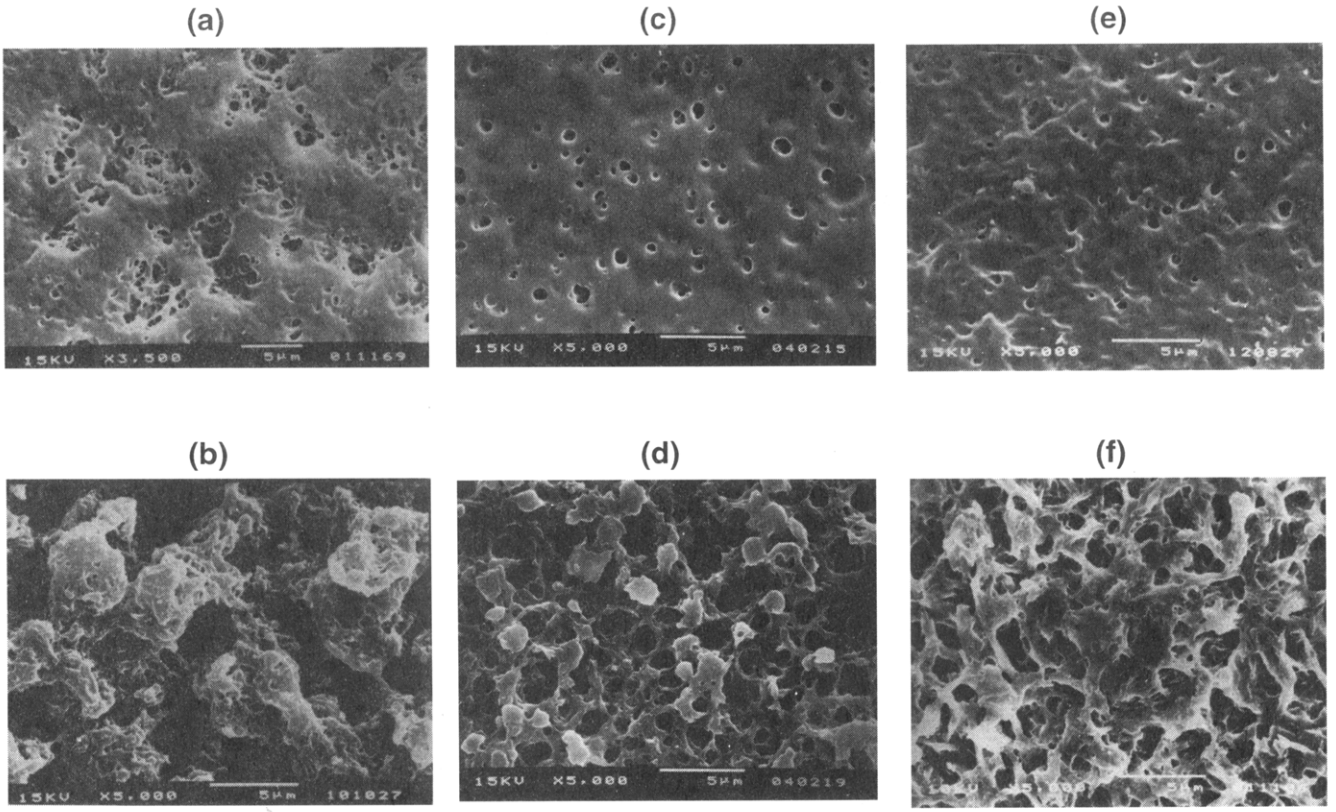

Figure 6. SEMs of surfaces of the blend films: P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)] (a) before and (b) after enzymatic degradation for 2 h; P[(*R*)-3HB]/P(6HH)/P(*R*,*S*)-3HB-*b*-6HH] [71/24/5 (wt ratio)] (c) before and (d) after enzymatic degradation for 2 h; P[(*R*)-3HB] homopolymer (e) before and (f) after enzymatic degradation for 2 h.

inactive for the hydrolysis of P(6HH). In the case of the ternary blend containing P[(*R*,*S*)-3HB-*b*-6HH], the film was degraded at the same rate as that of the P[(*R*)-3HB] homopolymer during the initial stage of degradation for 4 h. After degradation for 4 h, the rate of enzymatic degradation of the film was accelerated to the same rate as that of the binary blend of P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)].

Figure 6 shows the SEMS of surfaces of films of P[(*R*)-3HB]/P(6HH) [75/25 wt ratio)], P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] [71/24/5 (wt ratio)], and P[(*R*)-3HB] before and after enzymatic degradation for 2 h. In the SEM (Figure 6b) of the surface of the P[(*R*)-3HB]/P(6HH) [75/25 (wt ratio)] blend film after enzymatic degradation for 2 h, large P(6HH) particles of 5–10 μm in diameter and many large holes are observed as a result of macrophase separation in the immiscible blend. On the other hand, in the SEM (Figure 6d) of the surface of the P[(*R*)-3HB]/P(6HH)/P[(*R*,*S*)-3HB-*b*-6HH] [71/24/5 (wt ratio)] blend film after enzymatic degradation for 2 h, small P(6HH) particles of about 2 μm diameter are detected, and many small holes of about 2 μm in diameter are observed on the surface of the film. These results indicate that the size of P(6HH) particles in the blend films is apparently reduced in the presence of block copolymer. The size of P(6HH) particles in the SEMs is almost consistent with the size of P(6HH) dispersed domains observed in the phase-contrast optical micrographs (Figure 4). The surface of the P[(*R*)-3HB] film after enzymatic degradation for 2 h was blemished (see Figure 6f), since the rate of hydrolysis of P[(*R*)-3HB] chains in the amorphous state on the surface by PHB depolymerase is 20 times higher than that in the crystalline state.[37]

The enzymatic degradation of the P[(*R*)-3HB] film by PHB depolymerase occurs on the surface, and the rate is increased proportionally to the surface of film.[38] In a previous paper,[19] we reported that the surface of the immiscible blend films of P[(*R*)-3HB]/P(6HH) had many large holes as a result of macrophase separation and that the surface area of the immiscible blend film was increased by the formation of large holes. As a result, the rate of surface erosion of the immiscible P[(*R*)-3HB]/P(6HH) blend film by PHB depolymerase was higher than that of the P[(*R*)-3HB] film. The surface of the ternary blend film containing the P[(*R*,*S*)-3HB-*b*-6HH] block copolymer as a compatibilizer is apparently smooth (see Figure 6c), and the surface area of the film may be identical with that of the P[(*R*)-3HB] film. As a result, the erosion rate of the ternary blend film by PHB depolymerase was the same as that of P[(*R*)-3HB] film during the initial stage of enzymatic degradation. The spilling of enzymatically inactive P(6HH) particles from the surface occurred during the enzymatic degradation, resulting in an increase of surface area of the film. Then, the erosion of the ternary blend film by the PHB depolymerase may be accelerated.

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