1 **Transition of Spherulite Morphology in a Crystalline/Crystalline Binary Blend of**
2 **Biodegradable Microbial Polyesters**

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Abstract

Crystalline/crystalline binary blend films of microbial polyesters composed of poly[(*R*)-3-hydroxybutyrate-*co*-(*R*)-3hydroxyhexanoate] (P(3HB-*co*-3HH)) and poly[(*R*)-3-hydroxybutyrate] (P(3HB)) that exhibit a morphological change are prepared by solvent casting. Differential scanning calorimetry measurements indicate that P(3HB-*co*-3HH) and P(3HB) are miscible for all blend ratios because a single glass transition temperature is observed. Polarization optical microscopy is used to investigate the transition of spherulite morphology and measure the radial growth rate of spherulites in the blend films. P(3HB-*co*-3HH) and P(3HB) contain positive spherulites, whereas in the binary blends, spherulite morphology changes from positive to negative. This change is related to the different growth rates of P(3HB-*co*-3HH) and P(3HB) lamellar crystals. Partial enzymatic degradation of the film surfaces reveals that the lamellar crystals of negative spherulites are oriented both perpendicular and parallel to the radial direction of spherulites. A new growth mechanism for spherulites in crystalline/crystalline blends is constructed from the results obtained for the blend films.

Key word: Polyhydroxyalkanoate; Binary blend; Transition of Spherulite Morphology

1. Introduction

Poly[(*R*)-3-hydroxybutyrate] (P(3HB)) is produced from renewable resources such as sugar and plant oil by microbial organisms [1]. P(3HB) is a crystalline thermoplastic, and is biodegradable in natural environments [2]. Until recently, P(3HB) was thought impractical because of its weak, brittle mechanical properties [3, 4]. However, the mechanical properties of P(3HB) films and fibers have recently been improved by increasing its molecular weight[5-8] , developing new drawing methods [9-12], biosynthesis of P(3HB) copolymers [13-19] and blending P(3HB) with other polymers [20].

The brittleness of P(3HB) has been decreased by copolymerization with a second monomer, such as 3-hydroxyvalyrate [21], 3-hydroxyhexanoate [16, 18], and 4-hydroxybutyrate [14]. The second monomer decreases the crystallinity of P(3HB) and increases its flexibility. Poly[(*R*)-3-hydroxybutyrate-*co*- (*R*)-3-hydroxyhexanoate] (P(3HB-*co*-3HH)) is a copolymer of P(3HB) that is biosynthesized from palm oil [22]. The mechanical properties of P(3HB-*co*-3HH) were improved as the 3HH ratio was increased from 0% to 25%; for example, elongation at break increased from 5% to 850% [18]. Polymer blending is another method to improve the material properties of P(3HB). Blends of P(3HB) with other polymers such as poly(epichlorohydrin) [23], poly(*p*-vinylphenol) [24], cellulose ester [25, 26], poly(vinyl acetate) [27], atactic poly(3- hydroxybutyrate) [28], poly(lactic acid) [29, 30] and poly(ethylene oxide) [31, 32] have been reported. Investigation of the spherulitic morphology and highly-ordered structure of polymer blends is also important from the viewpoints of both fundamental science and industry. Feng *et al.* reported the melting temperature (T_m) and glass transition temperature (T_g) of P(3HB-*co*-3HH)/P(3HB) blends [33], but the crystalline and spherulitic morphologies, growth rate of spherulites and higher-order structures of P(3HB-*co*-3HH)/P(3HB) blends have not been investigated.

In this study, we found that P(3HB-*co*-3HH)/P(3HB) blends exhibit an interesting

spherulitic morphology with a negative or positive transition depending on blend ratio. We reveal the cause of this transition of spherulitic morphology by differential scanning calorimetry (DSC), polarization optical microscopy (POM), and wide- and small-angle X-ray measurements (WAXD and SAXS) of the blends. Furthermore, partial enzymatic degradation was used to visualize the orientation of lamellar crystals in negative and positive spherulites of blend films by scanning electron microscopy (SEM).

2. Experimental Section

2.1 Materials

Poly[(*R*)-3-hydroxybutyrate-*co*-9.6 mol%-(*R*)-3-hydroxyhexanoate] (P(3HB-*co*-3HH)) [weight-average molecular weight (M_w) = 2.6×10^5 , number-average molecular weight (M_n) = 1.5×10^5 and polydispersity (PDI) = 1.7] was supplied by Kaneka Co. . P(3HB) was supplied by ICI Co. [M_w = 5.2×10^5 , M_n = 3.3×10^5 and PDI = 1.6]. P(3HB-*co*-3HH) and P(3HB) were purified by reprecipitation from chloroform solution using *n*-hexane and then dried under vacuum at 30 °C for 3 days.

2.2 Preparation of binary blend films

Blend films were prepared by solvent-casting in chloroform using various blend ratios from 100/0 to 0/100 of P(3HB-*co*-3HH)/P(3HB). The two polymers were mixed in chloroform and then cast onto a Petri dish. The Petri dish was covered with aluminum foil containing air holes, and stored at room temperature for 3 days. Each film was completely dried after drying under vacuum for 3 days.

2.3 Thermal properties of blend films

The cold crystallization temperature (T_{cc}), T_g and T_m of the blend films were measured by

DSC (Perkin-Elmer, Inc., DSC-8500, USA). Temperature calibration of the instrument was performed using indium. DSC properties of all samples were measured under a nitrogen atmosphere. The first heating cycle involved heating at a rate of 20 °C/min from -40 °C to 200 °C, maintaining at 200 °C for 1 min, and then quenching to -40 °C at a rate of -200 °C/min. T_g , T_{cc} and T_m were determined from DSC curves obtained for the second heating cycle at a heating rate of 20 °C/min. The thermal properties of the blends after isothermal crystallization at 90 °C were measured by DSC to reveal their higher-order structure.

2.4 POM observation

Samples for morphological observation were prepared by melting blend films between two cover glasses in a washer with a thickness of 20 μ m. *In situ* observation of growing spherulites was carried out by POM (Nikon, Eclipse E600 POL, Japan) equipped with a temperature jump stage (two hot-stages) (Japan High Tech Co., LK-300B, Japan). The polarizer and depolarizer were kept at right angles. The first hot-stage was set at 200 °C, and other was set at the desired crystallization temperature. Each sample was melted at 200 °C for 30 s on the first hot-stage, and then the molten sample was transferred to the second hot-stage. Observation was continued until the field was completely covered with spherulites. The radial growth rate of spherulites was calculated from the slope of spherulite radius against observation time. Samples with blend ratios of 100/0, 90/10, 85/15, 75/25, 70/30, 60/40, 50/50, 40/60, 25/75 and 0/100, all of which were annealed at a temperature at 90 °C, were investigated.

2.5 WAXD and SAXS measurements

To investigate the higher-order structure of the blend films, WAXD and SAXS measurements were performed using synchrotron radiation X-rays at BL-45XU and BL-47XU of SPring-8, Harima, Japan. The wavelength, camera length, exposure time and detector used

were 0.09 nm, 145 mm (WAXD) or 2400 mm (SAXS), 22 ms (WAXD) or 1058 ms (SAXS) and CCD camera, respectively.

2.6 Enzymatic etching and surface observation of annealed blend films

The amorphous regions on the surfaces of the annealed samples were etched by enzymatic degradation with extracellular PHB depolymerase from *Ralstonia pickettii* T1 (200 µg/mL) and potassium phosphate buffer (pH 7.4, 1 mL). After deposition of a Pt coating, film surfaces were observed by SEM (Hitachi, FE-SEM S-4000, Japan).

3. Results and discussion

3.1 Thermal properties

DSC thermograms of neat P(3HB-co-3HH), neat P(3HB) and blend films with various blend ratios of P(3HB-co-3HH) to P(3HB) are shown in Figure 1. T_g , T_{cc} , and T_m of each sample are listed in Table 1. Neat P(3HB-co-3HH) and P(3HB) exhibited T_g at -1.5 and 2.1 °C, respectively. For the blend samples, T_g decreased slightly with increasing content of P(3HB-co-3HH). The blends are completely miscible because only one T_g was detected for each. The peak positions of T_{cc} decreased as the content of P(3HB) increased. In contrast, the peak area of T_{cc} (ΔH_{cc}) increased as the content of P(3HB) increased. As the content of P(3HB) increased, T_m was almost constant but ΔH_{cc} increased (Table 1). These results indicate that P(3HB) only crystallized in blend films containing over 15% P(3HB). No crystallization or melting were detected in blend films containing less than 10% P(3HB) because of the limited crystallization time.

Figure 1. DSC thermograms of P(3HB-co-3HHx)/P(3HB) blends with various blend ratios from the second heating run. The arrows indicate glass transition points.

Table 1

T_g , T_{cc} , T_m and ΔH_{cc} of P(3HB-co-3HHx), P(3HB) and its blends.

Sample P(3HB-co-3HHx)/P(3HB)	T_g [°C]	T_{cc} [°C]	T_m [°C]	ΔH_{cc} [J/g]
100/ 0	-1.5	-	-	-
95/ 5	-1.3	-	-	-
90/ 10	-1.4	-	-	-
85/ 15	-1.5	83	165	1.4
80/ 20	-1.3	85	168	15.1
75/ 25	-1.0	86	168	16.7
70/ 30	-0.9	89	167	29.1
50/ 50	-0.6	57	170	33.7
40/ 60	0.0	54	170	34.6
30/ 70	0.7	53	170	35.9
25/ 75	0.5	56	170	37.7
0/100	2.1	50	170	40.1

3.2 Thermal properties of annealed blend films

Figure 2 shows the DSC curves of the blend films crystallized isothermally at 90 °C until complete crystallization, which is when the field of the POM was filled with spherulites. The melting temperatures were listed at Table 2. The neat P(3HB) film exhibited a sharp melting peak at around 170 °C. The neat P(3HB-*co*-3HH) film showed broad melting peaks between 100 and 120 °C. The broadness of these peaks is caused by the different thicknesses of lamellar crystals. It is well known that the T_m of neat P(3HB-*co*-3HH) is lower than that of neat P(3HB) because microbial P(3HB-*co*-3HH) is random copolymer [34] and its 3HH unit is excluded from lamellar crystals of P(3HB) [18].

For the blend films, two melting peaks were observed around 110 and 170 °C corresponding to P(3HB-*co*-3HH) and P(3HB) crystals, respectively, for blend ratios of P(3HB-*co*-3HH)/P(3HB) from 95/5 to 80/20 (Table 2). This suggests that P(3HB) and P(3HB-*co*-3HH) crystallize separately in these blend films. One melting peak was observed at around 170 °C for P(3HB-*co*-3HH)/P(3HB) blend ratios from 75/25 to 0/100.

Figure 2. DSC thermograms of isothermally crystallized blend films. Isothermal temperature obtained from melts was 90 ° C.

Table 2
 T_m of P(3HB-co-3HH), P(3HB) and its blends
crystallized isothermally at 90 °C.

Sample P(3HB-co-3HH)/P(3HB)	T_m [°C]	$T_{1/2}$ [°C]
100/ 0	119	-
85/ 15	102	163
80/ 20	101	164
60/ 40	101	165
70/ 30	-	100
60/ 40	-	107
20/ 80	-	107
0/100	-	171

3.3 Morphological observation of blend films by POM

POM images of blend films annealed at 90 °C and schematic diagrams of the two types of spherulites are presented in Figure 3 and 4, respectively. When the first and third quadrants of a spherulite are blue, the spherulite is considered positive (Figure 4(a)). Conversely, when the second and fourth quadrants of a spherulite are blue, it is categorized as negative (Figure 4(b)). These differences depend on the alignment of the optical indicatrix parallel or perpendicular to the radial direction of a spherulite, as shown in the middle of Figure 4.

The spherulites of neat P(3HB-co-3HH) and P(3HB) are shown as positive (Figure 3(a) and (j), respectively). Because lamellar crystals stack perpendicular to the long axis of the optical indicatrix, the chain axes of P(3HB-co-3HH) and P(3HB) molecules align perpendicular to the radial direction of spherulites. This phenomenon was observed until the blend contained 50 % P(3HB) (Figure 3(g), (h) and (i)). However, when P(3HB-co-3HH) was blended into P(3HB) at blend ratios of 60 % (60/40) to 85 % (85/15), the optical morphology changed from positive to negative, as shown in Figure 3(c)-(f). This result indicates that the direction of the long axis of the indicatrix changed from parallel to perpendicular to the radial direction of spherulites. This is because parts of the lamellar crystals align along the radial direction in negative spherulites, as shown at the bottom of Figure 4 [35]. Positive spherulites were also observed for blend films

containing over 90% P(3HB-*co*-3HH) (Figure 3(b)). Table 3 shows the relationship between the morphologies of spherulites and P(3HB) content in the blend films. We believe that this is the first observation of this interesting behavior.

Figure 3. POM micrographs of isothermally crystallized samples at various ratios of P(3HB-*co*-3HH):P(3HB) (a)100/0 (-), (b)90/10 (-), (c)85/15 (-), (d)75/25 (-), (e)70/30 (-), (f)60/40 (-), (g)50/50 (-), (h)40/60 (-), (i)25/75 (-) and (j)0/100 (-). Scale bars are 500 μm . (-) and (+) indicate positive and negative spherulites, respectively.

Figure 4. Models of positive and negative spherulites of P(3HB). Normal spherulites of P(3HB-co-3HB) and P(3HB) are labeled “positive spherulites”.

Table3
Relationship between the morphologies of spherulites and P(3HB) content in blend films.

P(3HB) content (wt%)	0~10	15	20	25	30	40	50~100
Birefringence	+	-	-	-	-	-	+

+: Positive spherulite
- : Negative spherulite

3.4 Radial growth rate of spherulites

Figure 5 shows the radial growth rate (G) of spherulites in blend films against their content of P(3HB) during isothermal crystallization at 90 °C. The G value of neat P(3HB) was 0.25 mm/min, and complete crystallization took about 5 min. In contrast, G of neat P(3HB-co-3HH) was 0.0008 mm/min and 9 hours were required to fill the observation area with spherulites.

Figure 5. Spherulite growth rate in blend films isothermally crystallized at 90 ° C as a function of P(3HB) content.

The lamellar crystals in spherulites of P(3HB-co-3HH)/P(3HB) blend of 100/0 and 90/10 seem to be composed of P(3HB-co-3HH) chains, thus their G is extremely low. When the content of P(3HB) was over 15% (85/15) (phases 2 and 3), G increased considerably, suggesting that these spherulites consisted of P(3HB) crystals. This result supports the observation that the morphology of spherulites changed from positive to negative at a P(3HB) content of 15% (85/15), as shown in Figure 3.

G increased substantially as the content of P(3HB) increased from 15% (85/15) to 40% (60/40). The increase of G leveled off in the range of P(3HB) content from 50% (50/50) to 100% (0/100). The G values of the blends can be categorized into three phases.

Phase 1: very low G values depending on P(3HB-co-3HH) in P(3HB-co-3HH)/P(3HB) blends of 100/0-90/10. Phase 2: G increases drastically with increasing P(3HB) content in P(3HB-co-3HH)/P(3HB) blends of 85/15-60/40. Phase 3: G increased gradually with increasing P(3HB) content in P(3HB-co-3HH)/P(3HB) blends of 50/50-0/100. These phases correspond to the observed positive, negative and positive spherulite behavior (Figure 3).

3.5 SEM observation of enzymatically degraded blend films

The SEM observation was carried out to observe the growth morphology of lamellar crystal. Therefore, the observation areas were chosen the local field on the spherulite. It is well known that the enzymatic degradation of PHA progresses from amorphous to crystalline regions [36, 37]. Enzymatic edging was performed by stopping the enzymatic degradation after etching of amorphous regions. SEM images of the blend films are illustrated in Figure 6. In these SEM images, lamellar crystals grow from the left to right side (crystallographic a^* axis is on the right), so the left side shows the center of a spherulite. The rod-like crystals are stacked lamellar crystals. Lamellar crystals formed in neat P(3HB-co-3HH) (Figure 6(a)), but their orientation became disordered as the content of P(3HB) at 25% (Figure 6(b)). As the content of P(3HB) increased further, the morphology of lamellar crystals in spherulites again became similar to the initial aligned structure, as shown in Figure 6(c), (d) and (e).

The conversion between negative and positive morphologies in miscible blends of crystalline and amorphous polymers, such as blends of polyethylene oxide and polymethylmethacrylate, has been already reported[38]. However, this is the first observation of such behavior in crystalline/crystalline polymer blends. In the studies of spherulite crystals, such conversion indicates the formation of inordinate morphology caused by the increasing content of amorphous polymer [38] .

Figure 6. SEM images of isothermally crystallized blend films after etching by enzymatic degradation. Ratio of P(3HB-co-3HH)/P(3HB) (a)100/0, (b)75/25, (c)50/50, (d)25/75, and (e)0/100. Scale bars are 10 μm .

3.6 WAXD and SAXS measurements

WAXD and SAXS measurements were carried out to analyze the highly ordered structures of the blend films. It is well known that the growth direction of spherulites for P(3HB) and its copolymers is the a^* axis of lamellar crystals, as shown in Figure 7. Figure 8 shows the WAXS and SAXS patterns obtained for spherulites shown in Figure 6.

Figure 7. Relationship between the position of the incident X-ray on P(3HB-co-3HH) or P(3HB) spherulites and WAXD pattern.

Arc patterns appeared at equatorial and meridian lines in WAXD diagrams of the neat P(3HB-co-3HH), 50/50, 25/75 P(3HB-co-3HH)/P(3HB) blend and neat P(3HB) films (Figure 8A, D and E, respectively). This suggests that the lamellar crystals were oriented along the a^* axis [39, 40]. Furthermore, corresponding SAXS patterns indicate that lamellar crystals stacked perpendicular to the growth direction of spherulites.

In contrast, WAXD and SAXS patterns of the films with P(3HB-co-3HH)/P(3HB) blend ratios of 75/25 (Figure 8B) showed ring patterns, which indicates that lamellar crystals grew randomly against the radial direction of spherulites. These ring patterns were detected regardless of the position of the X-ray beam to the spherulites.

Figure 8. WAXD (A-E) and SAXS (a-e) patterns of isothermally crystallized blend films with ratios of P(3HB-co-3HH):P(3HB) of (A, a) 100/0, (B, b) 75/25, (C, c) 50/50, (D, d) 25/75, and (E, e) 0/100.

3.7 Generation mechanism for the conversion of spherulite morphology

We report here the first example of conversion between positive and negative spherulite morphologies in crystalline/crystalline polymer blends. Above, we categorized the G values of the films into three phases depending on the content of P(3HB), 0%–10% (100/0-90/10), 15%–40% (85/15-60/40), and 50%–100% (50/50-0/100) (Figure 5). These phases correspond to the spherulite morphologies of positive, negative, and positive, as shown in Figure 3.

A schematic illustration of the highly ordered structure of the blends in phases 1, 2, and 3 is presented in Figure 9. In phase 1 (P(3HB-*co*-3HH):P(3HB) of 100/0–90/10), the spherulites consist of P(3HB-*co*-3HH) lamellar crystals, whereas in phases 2 and 3 (P(3HB-*co*-3HH)/P(3HB) of 85/15–0/100), the spherulites consist of P(3HB) lamellar crystals. The difference between phase 2 and 3 is the kind of molecular chains present in amorphous regions. The amorphous regions in phase 2 consist of P(3HB-*co*-3HH) chains, whereas those in phase 3 are composed of P(3HB) chains.

In phase 1, ordered growth was observed in the radial direction (Figure 6(a) and 8A). In phase 3, P(3HB) crystals grew parallel to the radial direction. However, in phase 2, P(3HB) crystals seem to grow around P(3HB-*co*-3HH) amorphous chains. Accordingly, P(3HB) crystals grow both perpendicular and parallel to the radial direction. Such disordered growth of P(3HB) crystals was confirmed by the results of both SEM (Figure 6(b)) and WAXD (Figure 8B). The negative spherulites observed for phase 2 appear to be formed by the generation of P(3HB) crystals aligned perpendicular to the radial direction of spherulites. The transition of G value between phase 1 and 2 in Figure 5 possibly means that the growth mode of P(3HB) crystals changed from “ordered” to “disordered”. Thus, in positive spherulites, optical indicatrix all align parallel to the radial direction. However, in negative spherulites, optical indicatrix seem to align both parallel and perpendicular to the radial direction.

Figure 9. Schematic illustration of the highly ordered structures of crystallized P(3HB-co-3HH), P(3HB), and their blend films.

4. Conclusion

Microbial polyester blends of P(3HB-*co*-3HH) and P(3HB) were found to be miscible independent of blend ratio. POM observation found that some of the blends contained negative spherulites, whereas neat P(3HB-*co*-3HH) and P(3HB) has positive ones. The G values of the blends were categorized into three phases that corresponded to positive, negative and positive spherulite morphologies depending on the content of P(3HB) in the blend. Film surfaces observed after partial enzymatic etching showed that the orientation of lamellar crystals changed from ordered to disordered depending on P(3HB-*co*-3HH)/P(3HB) blend ratio. The appearance of negative spherulites in some blends seems to be caused by the existence of optical indicatrix that are parallel and perpendicular to the radial direction of spherulites. We proposed a new conversion mechanism between negative and positive spherulites in crystalline/crystalline polymer blends.

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472 **References**

- 473 1. Macrae RM and Wilkinson JF. *Journal of General Microbiology* 1958;19(1):210-222.
- 474 2. Lenz RW and Marchessault RH. *Biomacromolecules* 2005;6(1):1-8.
- 475 3. Scandola M, Ceccorulli G, and Pizzoli M. *Makromolekulare Chemie-Rapid*
476 *Communications* 1989;10(2):47-50.
- 477 4. Dekoning GJM and Lemstra PJ. *Polymer* 1993;34(19):4089-4094.
- 478 5. Kusaka S, Abe H, Lee SY, and Doi Y. *Applied Microbiology and Biotechnology*
479 1997;47(2):140-143.
- 480 6. Kusaka S, Iwata T, and Doi Y. *Journal of Macromolecular Science-Pure and Applied*
481 *Chemistry* 1998;A35(2):319-335.
- 482 7. Kabe T, Tsuge T, Kasuya K, Takemura A, Hikima T, Takata M, and Iwata T.
483 *Macromolecules* 2012;45(4):1858-1865.
- 484 8. Kabe T, Tsuge T, Hikima T, Takata M, Takemura A, and Iwata T. *Processing,*
485 *Mechanical Properties, and Structure Analysis of Melt-Spun Fibers of*
486 *P(3HB)/UHMW-P(3HB) Identical Blend. Biobased Monomers, Polymers, and*
487 *Materials*, vol. 1105: American Chemical Society, 2012. pp. 63-75.
- 488 9. Iwata T, Tsunoda K, Aoyagi Y, Kusaka S, Yonezawa N, and Doi Y. *Polymer*
489 *Degradation and Stability* 2003;79(2):217-224.
- 490 10. Tanaka T, Fujita M, Takeuchi A, Suzuki Y, Uesugi K, Ito K, Fujisawa T, Doi Y, and
491 Iwata T. *Macromolecules* 2006;39(8):2940-2946.
- 492 11. Aoyagi Y, Doi Y, and Iwata T. *Polymer Degradation and Stability* 2003;79(2):209-216.
- 493 12. Kabe T, Tanaka T, Kasuya K-i, Ito K, Takata M, Takemura A, and Iwata T. *Sen'i*
494 *Gakkaishi* 2010;66(10):253-260.
- 495 13. Bloembergen S, Holden DA, Hamer GK, Bluhm TL, and Marchessault RH.
496 *Macromolecules* 1986;19(11):2865-2871.
- 497 14. Doi Y, Kunioka M, Nakamura Y, and Soga K. *Macromolecules* 1988;21(9):2722-2727.
- 498 15. Hiramitsu M and Doi Y. *Polymer* 1993;34(22):4782-4786.
- 499 16. Liebergesell M, Mayer F, and Steinbuchel A. *Applied Microbiology and Biotechnology*
500 1993;40(2-3):292-300.
- 501 17. Kichise T, Fukui T, Yoshida Y, and Doi Y. *International Journal of Biological*
502 *Macromolecules* 1999;25(1-3):69-77.
- 503 18. Doi Y, Kitamura S, and Abe H. *Macromolecules* 1995;28(14):4822-4828.
- 504 19. Sudesh K, Abe H, and Doi Y. *Progress in Polymer Science* 2000;25(10):1503-1555.
- 505 20. Di Lorenzo ML. *Progress in Polymer Science* 2003;28(4):663-689.
- 506 21. Holmes PA. *Physics in Technology* 1985;16(1):32-36.
- 507 22. Loo C-Y, Lee W-H, Tsuge T, Doi Y, and Sudesh K. *Biotechnology Letters*
508 2005;27(18):1405-1410.
- 509 23. Finelli L, Sarti B, and Scandola M. *Journal of Macromolecular Science-Pure and*
510 *Applied Chemistry* 1997;A34(1):13-33.
- 511 24. Xing PX, Dong LS, An YX, Feng ZL, Avella M, and Martuscelli E. *Macromolecules*
512 1997;30(9):2726-2733.
- 513 25. Zhang L, Deng X, and Huang Z. *Polymer* 1997;38(21):5379-5387.
- 514 26. Pizzoli M, Scandola M, and Ceccorulli G. *Macromolecules* 1994;27(17):4755-4761.
- 515 27. Greco P and Martuscelli E. *Polymer* 1989;30(8):1475-1483.
- 516 28. Abe H, Doi Y, Satkowski MM, and Noda I. *Macromolecules* 1994;27(1):50-54.
- 517 29. Blümm E and Owen AJ. *Polymer* 1995;36(21):4077-4081.
- 518 30. Koyama N and Doi Y. *Polymer* 1997;38(7):1589-1593.
- 519 31. Avella M and Martuscelli E. *Polymer* 1988;29(10):1731-1737.
- 520 32. Kumagai Y and Doi Y. *Polymer Degradation and Stability* 1992;35(1):87-93.

- 521 33. Feng LD, Watanabe T, He Y, Wang Y, Kichise T, Fukuchi T, Chen GQ, Doi Y, and
522 Inoue Y. *Macromolecular Bioscience* 2003;3(6):310-319.
- 523 34. Shimamura E, Kasuya K, Kobayashi G, Shiotani T, Shima Y, and Doi Y.
524 *Macromolecules* 1994;27(3):878-880.
- 525 35. Norton DR and Keller A. *Polymer* 1985;26(5):704-716.
- 526 36. Kumagai Y, Kanesawa Y, and Doi Y. *Makromolekulare Chemie-Macromolecular*
527 *Chemistry and Physics* 1992;193(1):53-57.
- 528 37. Iwata T, Doi Y, Tanaka T, Akehata T, Shiromo M, and Teramachi S. *Macromolecules*
529 *1997;30(18):5290-5296.*
- 530 38. Shi W, Yang J, Zhang Y, Luo J, Liang Y, and Han CC. *Macromolecules*
531 *2012;45(2):941-950.*
- 532 39. Barham PJ, Keller A, Otun EL, and Holmes PA. *Journal of Materials Science*
533 *1984;19(9):2781-2794.*
- 534 40. Park JW, Tanaka T, Doi Y, and Iwata T. *Macromolecular Bioscience*
535 *2005;5(9):840-852.*
- 536
- 537

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Figure captions

Figure 1. DSC thermograms of P(3HB-*co*-3HHx)/P(3HB) blends with various blend ratios from the second heating run. The arrows indicate glass transition points.

Figure 2. DSC thermograms of isothermally crystallized blend films. Isothermal temperature obtained from melts was 90 °C.

Figure 3. POM micrographs of isothermally crystallized samples at various ratios of P(3HB-*co*-3HH:P(3HB) (a)100/0 (+), (b)90/10 (+), (c)85/15 (-), (d)75/25 (-), (e)70/30 (-), (f)60/40 (-), (g)50/50 (+), (h)40/60 (+), (i)25/75 (+) and (j)0/100 (+). Scale bars are 500 μm. (+) and (-) indicate positive and negative spherulites, respectively.

Figure 4. Models of positive and negative spherulites of P(3HB). Normal spherulites of P(3HB-*co*-3HB) and P(3HB) are labeled “positive spherulites”.

Figure 5. Spherulite growth rate in blend films isothermally crystallized at 90 °C as a function of P(3HB) content.

Figure 6. SEM images of isothermally crystallized blend films after etching by enzymatic degradation. Ratio of P(3HB-*co*-3HH/P(3HB) (a)100/0, (b)75/25, (c)50/50, (d)25/75, and (e)0/100. Scale bars are 10 μm.

Figure 7. Relationship between the position of the incident X-ray on P(3HB-*co*-3HH) or P(3HB) spherulites and WAXD pattern.

Figure 8. WAXD (A-E) and SAXS (a-e) patterns of isothermally crystallized blend films with ratios of P(3HB-*co*-3HH:P(3HB) of (A, a) 100/0, (B, b) 75/25, (C, c) 50/50, (D, d) 25/75, and (E, e) 0/100.

Figure 9. Schematic illustration of the highly ordered structures of crystallized P(3HB-*co*-3HH), P(3HB), and their blend films.

564 **Table captions**

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566 **Table 1** T_g , T_{cc} , T_m and ΔH_{cc} of P(3HB-*co*-3HH), P(3HB) and its blends.

567 **Table 2** T_m of P(3HB-*co*-3HH), P(3HB) and its blends crystallized isothermally at 90 °C.

568 **Table 3** Relationship between the morphologies of spherulites and P(3HB) content in blend
569 films.

570

Graphical Abstract