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# Surface melting and crystallization behavior of polyhydroxyalkanoates studied by attenuated total reflection infrared spectroscopy

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# Abstract

The surface melting and crystallization behavior of two biodegradable polyesters, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(HB-co-HHx)) (HHx = 12 mol%) and poly(3-hydroxybutyrate) (PHB), were investigated by using attenuated total reflection (ATR) and transmission infrared (IR) spectroscopy coupled with the generalized two-dimensional (2D) correlation analysis. IR bands in the C==O stretching vibration region were analyzed to explore the changes in crystallinity at the surface and those in the bulk of the polymer film samples during the melting and crystallization processes. Due to the intrinsic sub-micrometer sampling depth of the ATR technique, spectral information attributed to the chemical moieties situated at the surface region of the film samples can be observed. The present study revealed that the surface melting of P(HB-co-HHx) takes place through an intermediate state. The distribution of crystalline phase detected by the ATR technique and that detected by the transmission technique suggested that the polymer crystals tend to grow at the surface in a manner different from that in the bulk. It is very likely that the population of polymer crystals at the surface is higher than that in the bulk for both P(HB-co-HHx) and PHB. The time-dependent IR spectral variations for P(HB-co-HHx) and those for PHB indicated that P(HB-co-HHx) crystallizes much slower than PHB. This observation suggested that the HHx units incorporated in P(HB-co-HHx) markedly reduce not only the degree of crystallinity but also the crystallization rate of PHB homopolymer. © 2004 Elsevier Ltd. All rights reserved.

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# 1. Introduction

The surface layer of a polymer often exhibits different properties, e.g. morphology, composition, and structure, from the bulk material [1], and it sometimes determines the overall characteristic of the polymer. In applications of polymers to thin films and coatings, it is often the interfacial behavior that governs the material performance rather than the bulk. For biocompatible objects such as a polymeric scaffold, the tissue/polymer interface where a cellular adhesion occurs is also very important [2–4]. The biocompatibility depends strongly on the surface morphology of the polymer [2]. Consequently, the surface properties and surface phenomena of polymers are of great interest from both fundamental and technological points of views.

Crystallization behavior of macromolecules at the surface and interface regions plays an important role in numerous properties, e.g. mechanical strength, chemical compatibility, and biocompatibility, of polymeric materials [1,2]. Therefore, an insight into the surface crystallization behavior of polymers not only allows us to understand fundamental surface phenomena but also assists us to develop polymer products of high potential. In order to precisely extract the surface information, a non-destructive characterization technique capable of selectively probing surface molecules is required. There are various techniques

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applicable to polymer surface analysis, for examples, scanning electron microscopy (SEM) [2], X-ray photoelectron spectroscopy (XPS) [5], and atomic force microscopy (AFM) [5]. Attenuated total reflection (ATR) infrared (IR) spectroscopy is known as one of powerful surface characterization techniques that can provide information, e.g. chemical reactions, functional groups, and molecular orientation, at the surface and interface regions of polymers [6–11]. Quantitative information of a sample can also be obtained from a system with an optical contact between the sample and an internal reflection element (IRE) [8–11]. ATR IR spectroscopy possesses several advantages such as non-destructive nature, easy and fast operation, and little or no sample preparation [9–11].

In the present study, we have investigated the surface melting and crystallization behavior of two polyhydroxyalkanoate (PHA) polymers; poly(3-hydroxybutyrate-co-3hydroxyhexanoate) (P(HB-co-HHx)) (HHx = 12 mol%) and poly(3-hydroxybutyrate) (PHB). PHA polymers can be biologically derived from renewable carbon source like glucose or fatty acids and are subjected to degradation by bacteria in the soil [12-16]. Their chemical structures and physical characteristics are fairly similar to those of certain petroleum-based synthetic polymers [12–17]. Therefore, PHA polymers have received a great attention as an environmentally friendly polymeric material. PHB is the simplest and most abundant PHA polymer found in bacteria [12,15]. Since it is stiff and brittle due to the high degree of crystallinity [12–15], many attempts have been made to improve the mechanical properties of PHB [17]. It has been reported that P(HB-co-HHx) copolymers possess much better mechanical properties than PHB homopolymer [17, 18]. The applications of P(HB-co-HHx) copolymers, therefore, have been expanded to a greater extent including biodegradable films and coatings. From this aspect, the studies on the surface properties of this class of polymers are crucial not only for improving the performances of existing film products but also for developing new generation of biodegradable films.

We have conducted a series of studies on the structures and thermal behavior of PHB and P(HB-co-HHx) (12 mol% HHx) by using temperature-dependent wide-angle X-ray diffraction (WAXD) [19,20], differential scanning calorimetry (DSC) [19,20], and IR spectroscopy [20-22]. It was revealed by the previous studies that the phase transition process of P(HB-co-HHx) (12 mol% HHx) takes place through an intermediate state [22], and that the inclusion of the HHx comonomer locally disrupts the highly ordered and helical structure of PHB, thereby reducing the crystallinity of the PHB homopolymer without altering the crystalline structure [20,22]. The existence of an intermediate state during the phase transition of P(HB-co-HHx) (10 mol% HHx) and that of P(HB-co-HV) was also reported by Wu et al. [23] and Tian et al. [24], respectively. Recently, Kai et al. [2] investigated the effects of surface morphology on the biocompatibility of P(HB-co-HHx)/PHB blends by using SEM. It was found that the presence of P(HB-co-HHx) in the blends strongly improves the biocompatibility of PHB by providing the blending films with a fairly regular and smooth surface that allows cell attachment and growth. This study demonstrated an example of the influence of surface characteristics on the medical applications of P(HB-co-HHx) and PHB. However, the melting and crystallization behavior of P(HB-co-HHx) and PHB viewed at the film surface and interface regions have never been reported. The present study aims at investigating the melting and crystallization behavior of P(HB-co-HHx) copolymer and PHB homopolymer at the film/ATR crystal interface by using ATR and transmission IR spectroscopy coupled with the generalized two-dimensional (2D) correlation analysis. The time-dependent ATR and transmission spectra of P(HB-co-HHx) and those of PHB were monitored in order to follow the time-dependent crystal growth at the surface and in the bulk of the two polymers. The temperature-dependent IR spectral variations observed for the surface of a P(HB-co-HHx) film sample during the melting process were also compared to those observed for the bulk sample [22].

# 2. Experimental section

# 2.1. Materials

Bacterially synthesized P(HB-*co*-HHx) (12 mol% HHx) ( $T_{\rm m} \approx 110$  °C and ~35% crystallinity [18]) and PHB ( $T_{\rm m} \approx 170$  °C and ~55% crystallinity [17]) were provided by the Procter and Gamble Company, Cincinnati, USA. Purification of the P(HB-*co*-HHx) sample was performed by dissolving P(HB-*co*-HHx) in hot chloroform (CHCl<sub>3</sub>), reprecipitating in methanol (CH<sub>3</sub>OH), and vacuum-drying at 60 °C for 24 h. The CHCl<sub>3</sub>:CH<sub>3</sub>OH volume ratio for the reprecipitation was approximately 1:10. The PHB sample is a purified sample and, thus, was used as received. Chloroform and methanol were purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Chloroform was used as a solvent for P(HB-*co*-HHx) and PHB film castings.

# 2.2. IR spectroscopic measurements

#### 2.2.1. Temperature-dependent ATR measurements

A multiple reflection ATR accessory (Spectra Tech, USA) with a 45° ZnSe IRE  $(10 \times 75.5 \times 3 \text{ mm})$  was employed for the temperature-dependent ATR measurements. The temperature of the attachment that holds the ZnSe IRE with the P(HB-*co*-HHx) film sample was controlled by using a temperature controller unit (model LT230, CHINO). To assure the optical contact between the film sample and the IRE, the P(HB-*co*-HHx) film sample was prepared by casting its chloroform solution directly on the ZnSe IRE. The film was annealed at 60 °C in a vacuum-dried oven for 12 h and cooled down to room temperature (25 °C). Temperature-dependent ATR spectra of the film

sample were collected at a  $2 \text{ cm}^{-1}$  spectral resolution by using a Nicolet Magna-IR<sup>TM</sup> 550 spectrometer equipped with a mercury cadmium telluride (MCT) detector. A number-of-scan of 512 was accumulated to ensure a high signal-to-noise ratio. The measurements were carried out over a temperature range of 30–140 °C with an increment of 10 °C.

#### 2.2.2. Time-dependent IR measurements

The same ATR experimental set up as above without the temperature controller unit was employed for the time-dependent ATR measurements of P(HB-*co*-HHx) and PHB film samples. Time-dependent transmission measurements were also performed for P(HB-*co*-HHx) and PHB. The film samples were prepared by casting their chloroform solutions on ZnSe transmission windows in a same manner as that for the ATR measurements.

All time-dependent measurements were conducted at room temperature. After the solvent castings, the film samples were air-dried for 30 min before the measurements. The time-dependent IR spectra were collected every 15 min until the crystal growth was by and large completed (i.e. no significant spectral change was observed).

In order to directly compare the surface information obtained from the ATR technique with the bulk information obtained from the transmission technique, all ATR spectra were penetration depth-corrected to eliminate the artifact arising from the wavenumber-dependent sampling depth of the ATR technique [25]. It should be noted that the inherent wavenumber-dependent ATR spectral intensity is eliminated by this operation. However, the corrected ATR spectra still carry the surface information of the polymer film samples since the actual sampling depth of the entire spectra is up to a few micrometers from the sample surface while the transmission spectra carry the bulk information of the whole film samples.

# 2.3. 2D Correlation analysis

All IR spectra were baseline-corrected prior to the 2D correlation analysis. The calculations of 2D correlation spectra were performed by using the 2D-Pocha software composed by Daisuke Adachi (Kwansei-Gakuin University, Japan). Temperature-averaged and time-averaged spectra were used as the reference spectra for the 2D correlation calculations of the temperature- and time-dependent IR spectra, respectively.

# 3. Results and discussion

3.1. Temperature-dependent ATR spectral variations in the C=O stretching vibration region of P(HB-co-HHx) (12 mol% HHx) during the melting process

Fig. 1 shows temperature-dependent ATR spectra of

P(HB-*co*-HHx) in the 1780–1680 cm<sup>-1</sup> region where bands due to the C=O stretching modes are expected to appear. A sharp band at 1720 cm<sup>-1</sup> gradually decreases with increasing temperature while a broad band at 1731 cm<sup>-1</sup> increases. These spectral variations reflect the melting process of the copolymer. Although the relative intensity of bands in the C=O stretching vibration region is different, the temperature-dependent changes observed for the ATR measurement are similar to those previously observed for the transmission measurement [22]. In our previous paper [22], the C=O stretching band observed at ca. 1720 cm<sup>-1</sup> in the IR spectra of P(HB-*co*-HHx) was assigned to that of the highly ordered crystalline structure while that observed at ca. 1740 cm<sup>-1</sup> was attributed to the amorphous phase.

Fig. 2 shows ATR (solid line) and transmission (dotted line) spectra of the annealed P(HB-co-HHx) film sample in the C=O stretching vibration region measured at 30 °C. It can be seen that the major difference between the ATR and transmission spectra lies in the relative intensity of the highly ordered crystalline C=O band at ca.  $1720 \text{ cm}^{-1}$  and the amorphous C=O band at ca. 1740 cm<sup>-1</sup>; the band at ca.  $1720 \text{ cm}^{-1}$  in the ATR spectrum is more sharp and intense than that in the transmission spectrum. In general, the refractive index (RI) of organic compounds is  $1.5 \pm 0.2$  [8], and that of ZnSe is 2.4 [8]. Accordingly, for the present study, the penetration depth  $(d_p)$  calculated for the C=O stretching vibration region,  $d_p(1720 \text{ cm}^{-1})$  is 1.17 µm. Due to the shallow sampling depth of the ATR technique, the ATR spectra shown in Figs. 1 and 2 (solid line), therefore, contain only the spectral information associated with the chemical species situated just a few micrometers beyond the sample surface. Thus, it is very likely that the differences between the ATR and transmission spectra are due primarily to the different behavior of molecules located at the surface and in the bulk of the film sample. The intensity ratio of the highly ordered crystalline C=O band to the amorphous one for the ATR spectrum is much higher than that for the transmission spectrum (Fig. 2), suggesting that the



Fig. 1. Representative ATR spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C=O stretching vibration region (1780–1680 cm<sup>-1</sup>) measured over a temperature range of 30–140 °C. Arrows indicate the direction of absorbance changes as temperature increases.



Fig. 2. ATR (solid line) and transmission (dotted line) spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C=O stretching vibration region measured at 30 °C.

crystallinity of the polymer is much higher at the film surface than in the bulk. The broad feature at ca.  $1740 \text{ cm}^{-1}$  in the ATR spectrum exists only as a slope with weak intensity.

To extract more detailed information about the spectral variations induced by the temperature change, 2D correlation analysis was employed. Fig. 3(A) and (B) shows the synchronous and asynchronous 2D correlation spectra in the spectral region of  $1790-1670 \text{ cm}^{-1}$  generated from the temperature-dependent ATR spectra measured during the temperature increase in the range of 30-100 °C, respectively. The spectra measured above 100 °C were excluded in the calculation of the 2D spectra. A pair of negative cross peaks developed at 1736,  $1718 \text{ cm}^{-1}$  in the synchronous spectrum (Fig. 3(A)) indicates the opposite directions of the intensity variations in these two correlated bands. In the asynchronous spectrum (Fig. 3(B)), a pair of cross peaks appears at 1732, 1718 cm<sup>-1</sup>. The presence of asynchronous cross peaks indicates the out-of-phase changes in the intensity of the band at ca.  $1732 \text{ cm}^{-1}$  and that of the band at ca.  $1718 \text{ cm}^{-1}$ . This observation clearly suggests that the disappearance of the highly ordered crystalline component of the copolymer does not simultaneously result in the formation of the amorphous structure. That is, the surface melting of P(HB-co-HHx) takes place through an intermediate state. The existence of an intermediate state was also reported for the bulk melting process of P(HB-co-HHx) (12 mol% HHx) [22] and that of some other copolymers [23,24].

The 2D correlation spectra generated from the temperature-dependent ATR spectra also reveal that the spectral changes occurring at the surface region particularly involve the highly ordered crystalline component and the amorphous structure. On the other hand, 2D correlation spectra generated from the temperature-dependent transmission spectra of P(HB-*co*-HHx) in the C==O stretching vibration region clearly reveal that the bulk melting of P(HB-*co*-HHx) involves two crystalline components (i.e. the highly ordered crystalline component and the minor crystalline component



Fig. 3. Synchronous (A) and asynchronous (B) IR correlation spectra of P(HB-*co*-HHx) (12 mol% HHx) in the C=O stretching vibration region generated from the temperature-dependent ATR spectra measured over a temperature range of 30-100 °C.

with a less-ordered structure) and the amorphous phase [22]. These observations suggest that the polymer crystals tend to grow at the surface in a manner different from the bulk. It is very likely that the molecules situated at the surface region of the P(HB-*co*-HHx) film sample prefer the highly ordered molecular chain structure.

3.2. Time-dependent IR spectral variations in the C=O stretching vibration region of P(HB-co-HHx) (12 mol% HHx)

Fig. 4(A) and (B) shows time-dependent ATR and transmission spectra of P(HB-co-HHx) in the C=O stretching vibration region, respectively. The IR spectral changes induced by the time-dependent crystallization are clearly observed both from the ATR (A) and transmission (B) measurements. This is because the crystallization rate of P(HB-co-HHx) is slow, one can supercool the system away from the thermal equilibrium. Consequently, an amorphous



Fig. 4. Representative time-dependent ATR (A) and transmission (B) spectra of P(HB-co-HHx) (12 mol% HHx) in the C=O stretching vibration region measured at room temperature (25 °C). Arrows indicate the direction of absorbance changes as time increases.

sample can be obtained well below its apparent  $T_{\rm m}$ , and the time-dependent crystal growth can be monitored. The transition from the amorphous to the crystalline state causes the downward shift in the C==O stretching vibration region. However, the spectral features of the ATR spectra and those of the transmission spectra are substantially different from each other. The highly ordered crystalline C==O band observed in the transmission spectra is not so sharp and intense as that observed in the ATR spectra. In other words, the ATR spectra show higher crystalline characteristic while the transmission spectra manifest more of the amorphous feature.

A direct comparison between the ATR and transmission spectra measured under the same experimental conditions shows clear spectral differences. Fig. 5 depicts the ATR and transmission spectra measured at 30 min after the film casting (A) and those measured when the crystal growth was completed (B). The spectra shown in Fig. 5 clearly indicate that the surface and bulk chemical species exhibit different crystallization behavior. To minimize the effect of a localized anomalous refractive index dispersion results from absorption peaks, a Ge IRE with higher refractive index ( $RI_{Ge}$ =4.0) was employed. Fig. 6 shows ATR spectra of P(HB-*co*-HHx) measured with the Ge IRE under the same experimental conditions as those shown in Fig. 5. The ATR spectra taken with the Ge IRE also show markedly



Fig. 5. ATR (solid line) and transmission (dotted line) spectra of P(HB-co-HHx) (12 mol% HHx) in the C=O stretching vibration region: first (A) and last (B) spectra measured during the time-dependent IR measurements.

different characteristics from those of the transmission spectra. For the ATR spectra measured when the crystal growth was completed (Fig. 6 (solid line)), the highly ordered crystalline C=O band at  $1721 \text{ cm}^{-1}$  is very sharp and intense while the amorphous band at ca.  $1740 \text{ cm}^{-1}$  is very weak. The spectral features and peak positions are similar to those of the ATR spectra taken with the ZnSe IRE. This observation confirms the difference in the surface and bulk crystallinity of the P(HB-*co*-HHx) copolymer.



Fig. 6. ATR spectra of P(HB-co-HHx) (12 mol% HHx) in the C=O stretching vibration region measured at 30 min after the solvent casting (dotted line) and when the crystal growth was completed (solid line) by using the Ge IRE.

By comparing Figs. 2 and 5(B), the effect of the crystallization temperature on the crystal growth at the film surface and that in the bulk can be discussed. The highly ordered crystalline C=O band in the transmission spectrum of the annealed P(HB-*co*-HHx) sample (Fig. 2 (dotted line)) is more intense than that of the non-annealed P(HB-*co*-HHx) sample (Fig. 5(B) (dotted line)). This observation suggests that the crystallization in the bulk proceeds far better when the sample is annealed at an elevated temperature (60 °C). On the other hand, the crystallization at the film surface is not strongly affected by the temperature at which the crystallization takes place. As seen in Figs. 2 (solid line) and 5(B) (solid line), the overall ATR spectral features of the C=O stretching bands for both cases are very similar.

Fig. 7(A) and (B) shows the synchronous and asynchronous 2D correlation spectra in the spectral region of 1790- $1670 \text{ cm}^{-1}$  generated from the time-dependent ATR spectra, respectively. A pair of cross peaks developed at 1732, 1720 cm<sup>-1</sup> in the asynchronous spectrum (Fig. 7(B)) reveals that the time-dependent surface crystallization process of P(HB-co-HHx) also occurs through an intermediate state. The reorganization of the amorphous structure does not simultaneously result in the fully formed highly ordered crystalline component. Similar to the surface melting, the time-dependent surface crystallization of P(HB-co-HHx) mainly involves the highly ordered crystalline component and the amorphous structure. This observation also suggests that at the film surface, polymer molecules favor the highly ordered molecular chain structure.

Fig. 8(A) and (B) shows the synchronous and asynchronous 2D correlation spectra in the spectral region of 1790- $1670 \text{ cm}^{-1}$  generated from the time-dependent transmission spectra, respectively. Two pairs of cross peaks develop at (1742, 1722) and (1728, 1722) cm<sup>-1</sup> in the asynchronous spectrum (Fig. 8(B)). The presence of asynchronous cross peaks indicates that the time-dependent bulk crystallization process of P(HB-co-HHx) occurs through an intermediate state. Note that, similar to the bulk melting process of P(HB-co-HHx) [22], two crystalline C=O bands (i.e. bands at ca. 1728 and 1722 cm<sup>-1</sup>) appear in the 2D correlation spectra generated from the timedependent transmission spectra. This observation suggests that the time-dependent crystallization occurring in the bulk of the film sample also involves two crystalline components and the amorphous phase.

# 3.3. Comparisons between the time-dependent crystal growth of P(HB-co-HHx) (12 mol% HHx) and PHB

It is known that the introduction of HHx units into the molecular chain of PHB results in dramatically improved mechanical properties of the resulting P(HB-*co*-HHx) copolymer [17]. The HHx units act as a less crystallizable part and thereby depress the degree of crystallinity of the



Fig. 7. Synchronous (A) and asynchronous (B) correlation spectra of P(HBco-HHx) (12 mol% HHx) in the C=O stretching vibration region generated from the time-dependent ATR spectra shown in Fig. 4(A).

PHB homopolymer significantly [17]. From this aspect, effects of the HHx units on the crystallization process of the PHB homopolymer are very interesting.

Fig. 9(A) and (B) shows time-dependent ATR and transmission spectra of PHB in the C=O stretching vibration region, respectively. The observed IR spectra again indicate the different crystallization behavior at the surface and in the bulk of the PHB film sample. As expected, the ATR spectra of PHB (Fig. 9(A)) strongly represent the crystalline characteristic, and the amorphous feature at ca. 1740 cm<sup>-1</sup> is very weak. Similar to P(HB-*co*-HHx), transmission spectra of PHB show higher amorphous characteristic as compared to the ATR spectra. However, time-dependent IR spectral variations for PHB shown in Fig. 9(A) and (B) are different from those for P(HB-co-HHx) shown in Fig. 4(A) and (B). In chloroform solution, both IR spectra of P(HB-co-HHx) and PHB show fully random-coil feature (see Fig. 10). After chloroform evaporated, P(HB-co-HHx) and PHB started to crystallize. However, we could not observe the IR spectra of the fully



Fig. 8. Synchronous (A) and asynchronous (B) correlation spectra of P(HBco-HHx) (12 mol% HHx) in the C=O stretching vibration region generated from the time-dependent transmission spectra shown in Fig. 4(B).

random-coil PHB for the solvent-free state. PHB assumes a crystalline state right after the solvent evaporation. This observation indicates that PHB crystallizes rapidly, much faster than P(HB-*co*-HHx). Although the highly ordered crystalline C==O band at ca. 1720 cm<sup>-1</sup> continues to develop as time passes, the overall spectral changes occurred during the time-dependent crystal growth are not pronounced. That is to say, PHB crystallizes at a much higher rate than P(HB-*co*-HHx). In other words, the HHx comonomer incorporated in P(HB-*co*-HHx) significantly reduces the crystallization rate of the PHB homopolymer. This is because the HHx unit is less crystallizable, and the presence of the HHx units in the PHB molecular chains may encumber the crystallization of the polymer from place to place.

### 4. Conclusion

The melting behavior at the surface of P(HB-*co*-HHx) (12 mol% HHx) film sample was investigated by using ATR



Fig. 9. Representative time-dependent ATR (A) and transmission (B) spectra of PHB in the C=O stretching vibration region measured at room temperature. Arrows indicate the direction of absorbance changes as time increases.

IR spectroscopy and the generalized 2D correlation analysis. The ATR technique is capable of probing the chemical moieties situated just a few micrometers beyond the sample surface. The 2D correlation spectra generated from the temperature-dependent ATR spectra in the C=O stretching vibration region suggest that the disappearance of the highly ordered crystalline component at the film surface does not simultaneously result in the formation of the amorphous structure. Similar to the bulk melting of P(HB*co*-HHx), the surface melting of P(HB-*co*-HHx) takes place



Fig. 10. Transmission spectra of P(HB-co-HHx) (12 mol% HHx) (solid line) and PHB (dotted line) in CHCl<sub>3</sub> solution.

through an intermediate state. However, the surface melting of P(HB-*co*-HHx) involves particularly the highly ordered crystalline component and the amorphous phase while the bulk melting involves two crystalline components (i.e. the highly ordered crystalline component and the minor crystalline component with a less-ordered structure) and the amorphous phase.

The time-dependent crystallization at the surface and bulk of P(HB-co-HHx) and PHB film samples was monitored by using ATR and transmission IR spectroscopy, respectively. It was revealed by the 2D correlation spectra generated from the time-dependent IR spectra of P(HB-co-HHx) in the C=O stretching vibration region that the surface and bulk crystal growth of P(HB-co-HHx) film also occurs through an intermediate state. Time-dependent ATR and transmission spectra of PHB indicate that PHB crystallizes at a much higher rate than P(HB-co-HHx). This observation suggests that the HHx units incorporated in the P(HB-co-HHx) copolymer significantly reduce not only the degree of crystallinity but also the crystallization rate of the PHB homopolymer. For both P(HB-co-HHx) and PHB film samples, it is very likely that the population of polymer crystals at the surface is higher than that in the bulk.

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