

## X-ray Diffraction and Infrared Spectroscopy Studies on Crystal and Lamellar Structure and CHO Hydrogen Bonding of Biodegradable Poly(hydroxyalkanoate)

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**Abstract:** Temperature-dependent, wide-angle, x-ray diffraction (WAXD) patterns and infrared (IR) spectra were measured for biodegradable poly(3-hydroxybutyrate) (PHB) and its copolymers, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) P(HB-co-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%), in order to explore their crystal and lamellar structure and their pattern of C-H $\cdots$ O=C hydrogen bonding. The WAXD patterns showed that the P(HB-co-HHx) copolymers have the same orthorhombic system as PHB. It was found from the temperature-dependent WAXD measurements of PHB and P(HB-co-HHx) that the *a* lattice parameter is more enlarged than the *b* lattice parameter during heating and that only the *a* lattice parameter shows reversibility during both heating and cooling processes. These observations suggest that an interaction occurs along the *a* axis in PHB and P(HB-co-HHx). This interaction seems to be due to an intermolecular C-H $\cdots$ O=C hydrogen bonding between the C=O group in one helical structure and the CH<sub>3</sub> group in the other helical structure. The x-ray crystallographic data of PHB showed that the distance between the O atom of the C=O group in one helical structure and the H atom of one of the three C-H bonds of the CH<sub>3</sub> group in the other helix structure is 2.63 Å, which is significantly shorter than the sum of the van der Waals separation (2.72 Å). This result and the appearance of the CH<sub>3</sub> asymmetric stretching band at 3009 cm<sup>-1</sup> suggest that there is a C-H $\cdots$ O=C hydrogen bond between the C=O group and the CH<sub>3</sub> group in PHB and P(HB-co-HHx). The temperature-dependent WAXD and IR measurements revealed that the crystallinity of P(HB-co-HHx) (HHx=10.5 and 12 mol%) decreases gradually from a fairly low temperature, while that of PHB and P(HB-co-HHx) (HHx=2.5 and 3.5 mol%) remains almost unchanged until just below their melting temperatures. It was also shown from our studies that the weakening of the C-H $\cdots$ O=C interaction starts from just above room temperature and proceeds gradually increasing temperature. It seems that the C-H $\cdots$ O=C hydrogen bonding stabilizes the chain holding in the lamellar structure and affects the thermal behaviour of PHB and its copolymers.

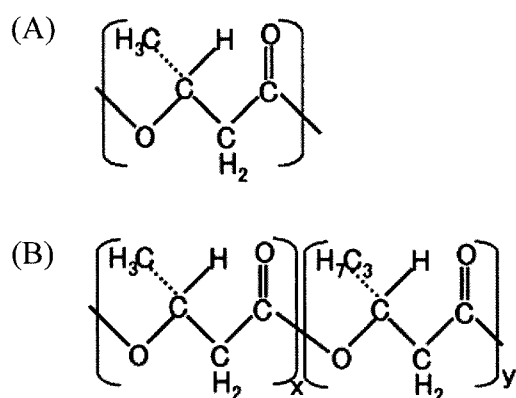
**Keywords:** poly(3-hydroxybutyrate), hydrogen bonding, lamellar structure.

### Introduction

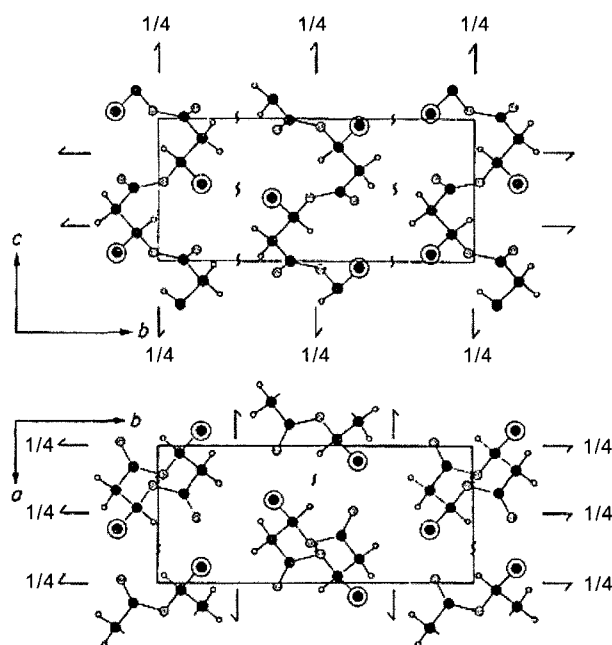
Poly(3-hydroxybutyrate) (PHB) and its copolymers, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), P(HB-co-HHx) (Figure 1) are biologically synthesized polyesters produced by bacteria.<sup>1-5</sup> These polymers are biodegradable thermo-

plastic and show good mechanical properties.<sup>6-8</sup> PHB is one of the most well-studied bacterial polyesters, and its structure and physical properties have been investigated by several research groups.<sup>8-15</sup> The crystal structure of PHB was investigated by x-ray crystallographic analysis, and it was found that PHB has an orthorhombic system, *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>(*D*<sub>2</sub><sup>4</sup>) with a fiber repeat of 5.96 Å (Figure 2).<sup>14,15</sup> PHB is rigid and stiff because of its high crystallinity from the perfect stereo-

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**Figure 1.** Chemical structures of (A) poly(3-hydroxybutyrate), PHB and (B) poly(3-hydroxybutyrate-co-hydroxyhexanoate), P(HB-co-HHx).



**Figure 2.** Crystalline structure of PHB (Reproduced from ref.15 with permission. Copyright (1972) Elsevier). (⊙)CH<sub>3</sub>; (⊙)C; (●)O; (○)H.

regularity produced by bacteria. In order to reduce the excess crystallinity and modify the overall physical properties of PHB, other monomers are sometimes copolymerized with PHB.<sup>13</sup> The PHB-based copolymers show a wide range of physical properties depending on the chemical structure of the comonomer units, as well as the comonomer composition.<sup>16</sup>

Recently, the Procter and Gamble Company (Cincinnati, USA) has introduced a new kind of PHB copolymers with a small amount of alkyl side chains, which has been commer-

cialized under the trade name *Nodax*<sup>TM</sup>.<sup>17</sup> P(HB-co-HHx) copolymer has a propyl side chain and it shows flexibility and good compatibility with other polymers. The biodegradation mechanism, chemical synthesis, and mechanical properties of P(HB-co-HHx) were reported by several research groups,<sup>18-21</sup> and its thermal behavior was investigated by differential scanning calorimetry (DSC).<sup>20,22</sup> The wide angle x-ray diffraction (WAXD) measurements of PHB and its copolymers were carried out at room temperature to characterize their crystallinity and crystal structure.<sup>20,21</sup> However, molecular interactions in their crystal and lamellar structure and thermal behaviour have not been explored well. Therefore, we started the studies on lamellar structure, crystallinity, molecular interactions and thermal behaviour of PHB and its copolymers by using WAXD,<sup>22,25</sup> infrared (IR) spectroscopy,<sup>23-25</sup> and quantum chemical calculation.<sup>24</sup>

One of the most important findings in our studies is the existence of the C-H...O=C hydrogen bond between the CH<sub>3</sub> group and the C=O group that combines the two parallel helical structures in PHB and P(HB-co-HHx).<sup>22-25</sup> Another important point of our studies is the effects of the addition of the HHx groups on the structure, crystallinity and thermal behavior of PHB and P(HB-co-HHx).<sup>22-25</sup> The WAXD studies also elucidated that P(HB-co-HHx) (HHx=12 mol%) shows largely different thermal behavior from that of PHB; the crystallinity of PHB remains almost unchanged until its melting point but that of P(HB-co-HHx) (HHx=12 mol%) decreases gradually from a fairly low temperature (~50°C). The *a* lattice parameter shows the thermal expansion, while the *b* lattice parameter shows very little change for PHB and only a very slight change for P(HB-co-HHx).<sup>22</sup> This result suggested that there is an inter- and intramolecular interaction between the C=O group and the CH<sub>3</sub> group in the P(HB-co-HHx) and PHB crystallinities and that the interaction becomes weak gradually with temperature along the *a* axis. In the IR studies of PHB and P(HB-co-HHx) we made band assignments in the C-H stretching, C=O stretching, and C-O-C stretching band regions and classified bands in the three regions into the crystalline bands and the amorphous bands. The anomalous high frequencies of CH<sub>3</sub> asymmetric stretching bands indicated the existence of the C-H...O=C hydrogen bonds in the PHAs investigated.<sup>22-25</sup> It was found from the temperature-dependent variations in the C-O-C stretching region of IR spectra that the changes in the helical structure start from a fairly low temperature and proceed gradually in P(HB-co-HHx) (HHx=12 mol%), while those occur rather suddenly just below the melting temperatures in PHB and P(HB-co-HHx) (HHx=2.5 and 3.4 mol%).

The purpose of this review paper is to report our recent WAXD and IR studies of PHB and P(HB-co-HHx).<sup>22-25</sup> In this review particular attention is paid to the C-H...O=C hydrogen bonds and thermal behavior of the polymers. To our knowledge, it is the first time that the existence of the

C-H...O=C hydrogen bondings has ever been demonstrated for polymers. Thus, our studies may provide new insight into weak interactions in polymers. In addition to the WAXD and IR data, new results for DSC and polarization optical microscope (POM) are presented in this article to provide further insight into the thermal properties, crystal and lamellar structure, and C-H...O=C hydrogen bond in PHB and P(HB-*co*-HHx) (HHx = 2.5, 3.4, 10.5 and 12 mol%).

## Experimental

**Samples.** Bacterially synthesized PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%) were provided from the Procter and Gamble Co., USA and Kaneka Co., Japan. The PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%) films for IR measurements were cast on CaF<sub>2</sub> windows from their chloroform solutions. The films were left overnight at 60 °C in a vacuum-dried oven.

**Wide Angle X-ray Diffraction (WAXD).** The temperature-dependent WAXD data were measured for the precipitated powder samples of PHB and P(HB-*co*-HHx) over a temperature range from room temperature to a high temperature in the scattering angle range of  $2\theta=5\text{--}13^\circ$  by using a two-circle Rigaku x-ray diffractometer equipped with a scintillation detector (RINT2000/PC). Mo-K $\alpha$  radiation (wavelength, 0.71069 Å) was used as an incident x-ray source (40 kV, 240 mA).

**IR Measurements.** IR spectra of the film samples were measured at a 2 cm<sup>-1</sup> spectral resolution by using a Thermo Nicolet NEXUS 470 Fourier transform IR spectrometer with a liquid-nitrogen-cooled mercury-cadmium-telluride detector. To ensure a high signal-to-noise ratio, 512 scans were co-added. Temperature of the films was controlled by a temperature controller unit (CHINO, model SU). The heating rate of the samples was ca. 2 °C min<sup>-1</sup>.

**Differential Scanning Calorimetry (DSC).** Differential scanning calorimetry (DSC) measurements of PHB and P(HB-*co*-HHx) were performed with a Perkin Elmer Pyris 6 DSC over a temperature range from 25 to 200 °C for PHB, to 180 °C for P(HB-*co*-HHx) (HHx=2.5 mol%), to 180 °C for P(HB-*co*-HHx) (HHx=3.4 mol%), and to 150 °C for P(HB-*co*-HHx) (HHx=12 mol%) at heating and cooling rates of 2 °C min<sup>-1</sup>, respectively.

**Polarization Optical Microscope (POM).** POM were measured for powder and film samples of PHB and P(HB-*co*-HHx) (HHx=12 mol%) by Nikon Polarization Microscope with a digital camera and a Metorer temperature contoroler over a temperature range from 25 to 200 °C for PHB and from 25 to 150 °C for P(HB-*co*-HHx) (HHx=12 mol%).

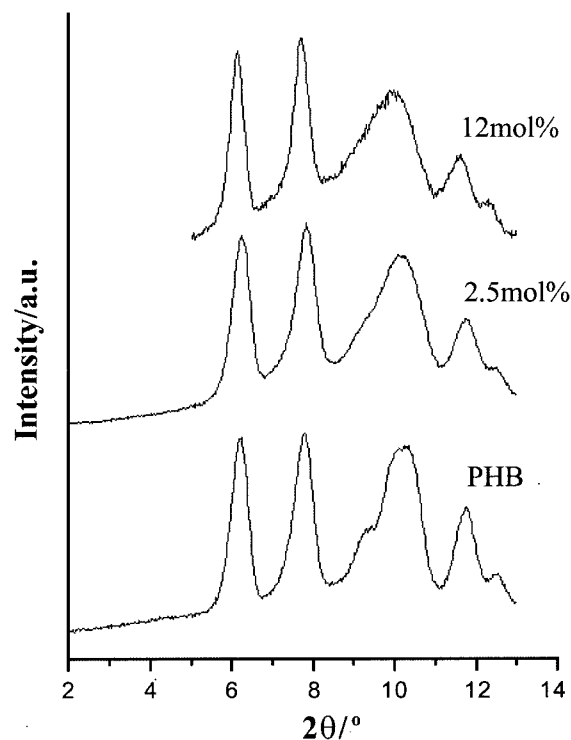
## Results and Discussion

**WAXD.** Figure 2 shows a crystalline structure of PHB

studied by Cornibert and Marchessault.<sup>15</sup> It was reported that the unit cells of PHB belong to the orthorhombic system,  $P2_12_12_1(D_2^4)$ , ( $\alpha=\beta=\gamma=90^\circ$ ) with  $a=5.76$  Å,  $b=13.20$  Å,  $c=5.96$  Å (fiber repeat).<sup>14,15</sup> Of note in Figure 2 is that the CH<sub>3</sub> group of PHB is close to the O atom of the C=O group on the  $a$  axis. We estimated the distance between the O atom of the C=O group in one helical structure and the H atom of one of the three C-H bonds of the CH<sub>3</sub> group in the other helix by using the x-ray crystallographic data of PHB.<sup>23,24</sup> In this calculation, we rotated the CH<sub>3</sub> group to find the shortest distance between the O and H atoms. The shortest distance was found to be 2.63 Å, which is significantly shorter than the sum of van der Waals separation (2.72 Å).<sup>23,24</sup>

Figure 3 shows x-ray diffraction patterns of PHB and P(HB-*co*-HHx) (HHx=2.5 and 12 mol%) at room temperature. It can be seen from this figure that the crystallinity decrease with the increases in the HHx content. The propyl side chains of the HHx units expand the  $a$  and  $b$  lattice parameters of PHB lattice in P(HB-*co*-HHx). Table I summarizes the  $a$  and  $b$  lattice parameters of PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%) at room temperature. The  $a$  and  $b$  lattice parameters increase with the increase in the HHx content.

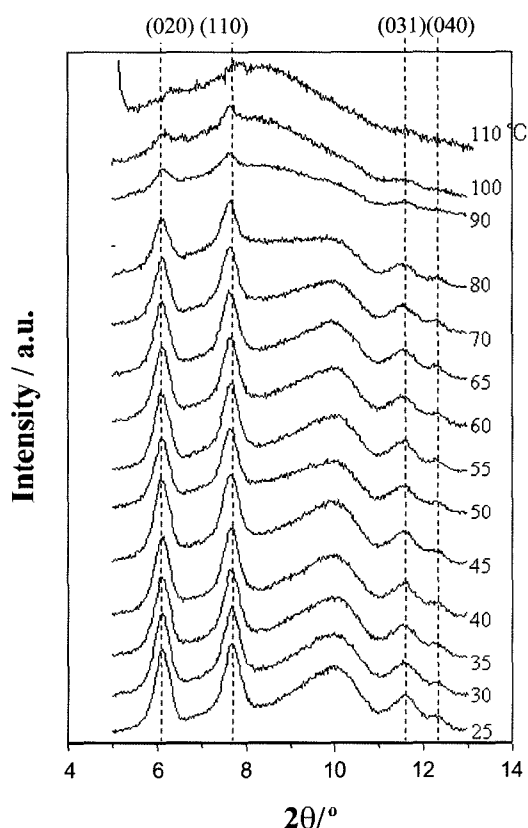
To explore the crystal and lamellar structure and the C-H...O=C hydrogen bond, temperature-dependent WAXD patterns were measured for PHB and P(HB-*co*-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%). Figure 4 shows the



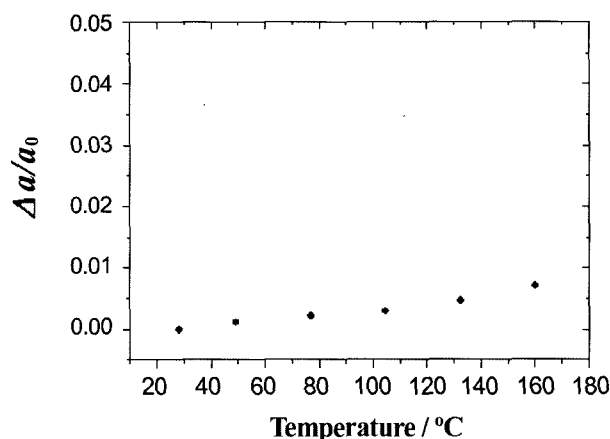
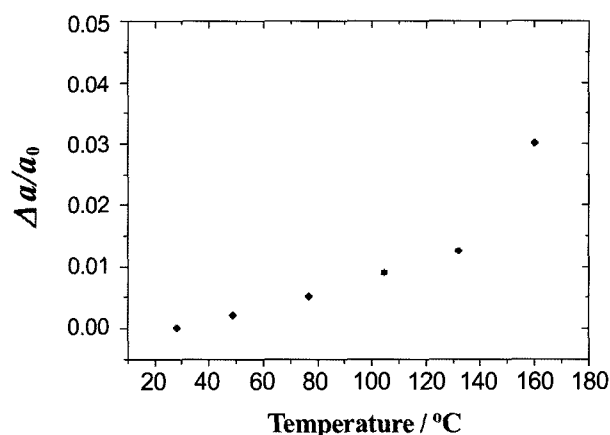
**Figure 3.** X-ray diffraction patterns of PHB and P(HB-*co*-HHx) (HHx=2.5 and 12 mol%) at room temperature.

**Table I. The Lattice Parameters of PHB and P(HB-co-HHx)**

Samples	$a/\text{\AA}$	$b/\text{\AA}$
PHB	5.74	13.19
P(HB-co-HHx) (HHx=2.5 mol%)	5.75	13.23
P(HB-co-HHx) (HHx=3.4 mol%)	5.80	13.47
P(HB-co-HHx) (HHx=10.5 mol%)	5.83	13.31
P(HB-co-HHx) (HHx=12 mol%)	5.76	13.33


**Figure 4.** Temperature dependence of the X-ray diffraction of P(HB-co-HHx) (HHx=2.5 mol%). (Reproduced from Ref. 22 with permission. Copyright (2004) American Chemical Society).

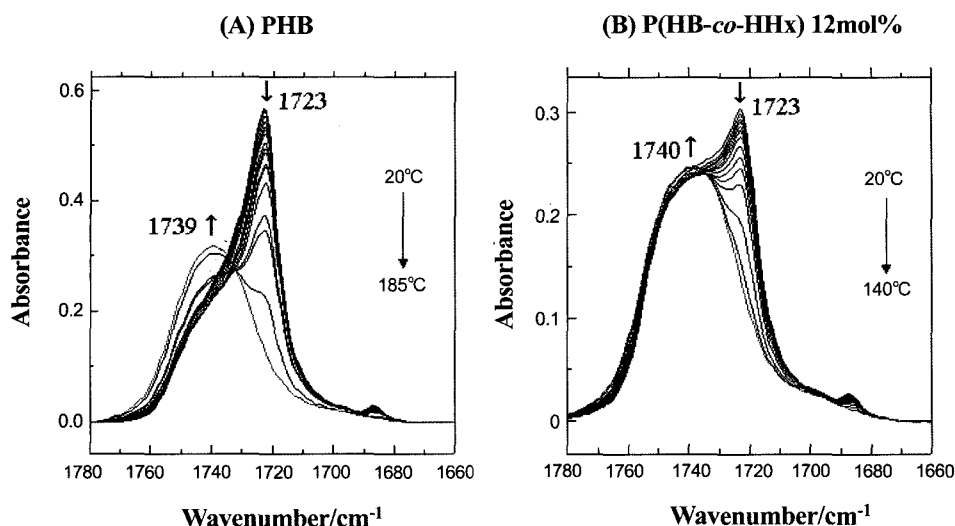
temperature dependence of x-ray diffraction of P(HB-co-HHx) (HHx=2.5 mol%) powder sample measured over a temperature range from 25 to 110 °C. It can be seen from Figure 4 that the (110)  $d$  spacing of P(HB-co-HHx) lattice expands as temperature increases. However, the (020)  $d$  spacing changes little with temperature. The WAXD patterns in Figure 4 show that the only  $a$  lattice parameter changes with temperature in the crystal lattice of P(HB-co-HHx). This result leads us to conclude that there is an intermolecular interaction between the C=O group and the CH<sub>3</sub>


**Figure 5.** Temperature-dependent variations in the increasing ratios of the lattice parameters,  $a$  and  $b$ , of PHB in the heating process.

group along the  $a$  lattice.

Figure 5 shows the increasing rates of the  $a$  and  $b$  lattice parameters for PHB versus temperature. The increasing rate of the  $a$  lattice parameter is larger than that of the  $b$  lattice parameter. Similar results were observed for P(HB-co-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%). Recently, Marchessault *et al.*<sup>26</sup> suggested based on the molecular weight studies on depolymerase-treated single crystals of PHB that the polymer chain folds along the  $a$  axis in the PHB single crystal. This specific chain folding of PHB may be the reason why the thermal expansion is much larger in the  $a$  lattice parameter than in the  $b$  lattice parameter.

**IR Spectroscopy.** Figure 6(A) and (B) displays temperature-dependent IR spectral variations in the C=O stretching band region of PHB and P(HB-co-HHx) (HHx=12 mol%), respectively. A band at 1723 cm<sup>-1</sup> is assigned to the C=O stretching mode of the crystalline parts while that around 1740 cm<sup>-1</sup> is due to the C=O stretching mode of the amorphous parts.<sup>23</sup> The temperature-dependent variations in the normalized peak height of the crystalline band at 1723 cm<sup>-1</sup> of PHB and P(HB-co-HHx) (HHx=12 mol%) during heating

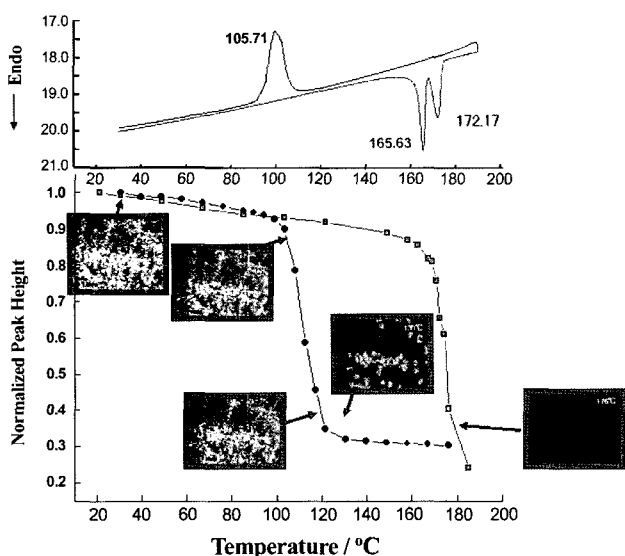


**Figure 6.** Temperature-dependent IR spectral variations in the C=O stretching band region of (A) PHB and (B) P(HB-co-HHx) (HHx=12 mol%). The spectra were measured over a temperature range of 20 °C to a high temperature (PHB, 185 °C; HHx=12 mol%, 140 °C). (Reproduced from Ref. 23 with permission. Copyright (2004) American Chemical Society).

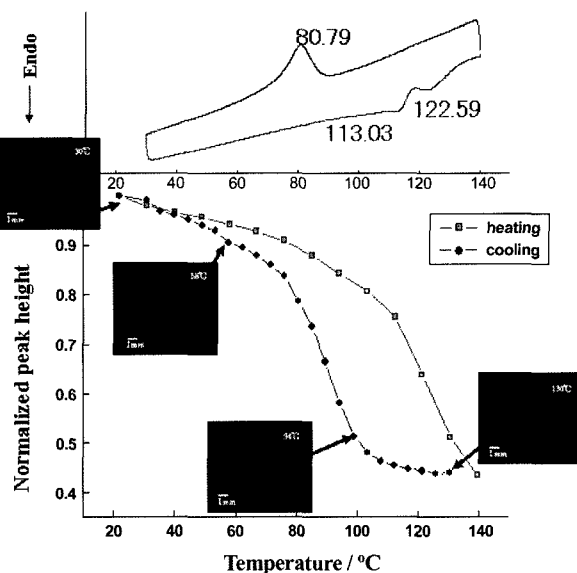
and cooling processes are shown in Figures 7 and 8, respectively. The crystalline band of PHB suddenly decreases around the melting temperature in the heating process, while that of P(HB-co-HHx) (HHx=12 mol%) shows a gradually change with the increase in temperature. These results indicate that the crystallinity of PHB remains almost unchanged until 160 °C, but that of P(HB-co-HHx) decreases gradually from fairly low temperature (~60 °C).<sup>23</sup>

Figures 7 and 8 also show the results for POM and DSC measurements. Note that the PHB sample gives clear spher-

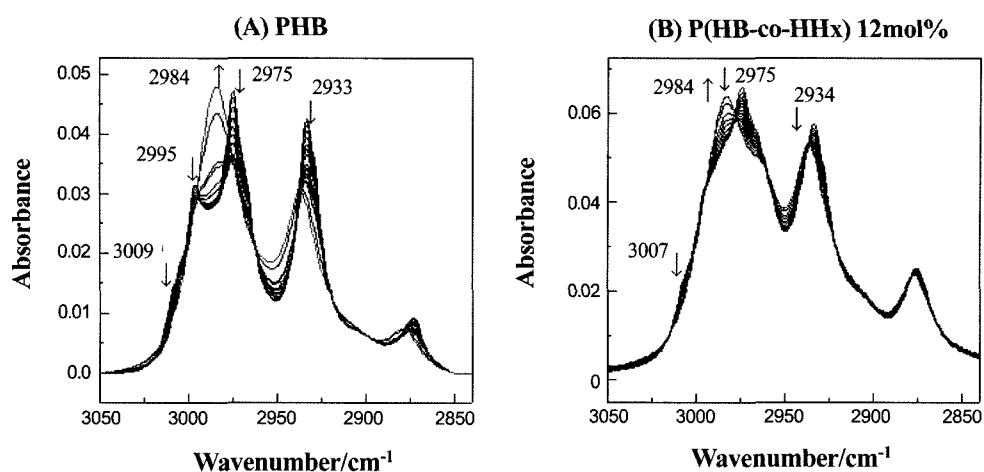
ulite structures in the POM image at room temperature. The spherulite structures of PHB remain until just below the melting point and suddenly disappear above it. In the cooling process, the spherulite structure appears at about  $T_c$ . These results are in good agreement with the DSC results. The color of the spherulites in the POM image of P(HB-co-HHx) (HHx=12 mol%) is changed gradually with the increase in temperature.



**Figure 7.** The normalized peak height of the crystalline band at 1723 cm<sup>-1</sup> in the C=O stretching band region versus temperature together with the results of POM image and DSC for PHB.



**Figure 8.** The normalized peak height of the crystalline band at 1723 cm<sup>-1</sup> in the C=O stretching band region versus temperature together with the results of POM image and DSC for P(HB-co-HHx) (HHx=12 mol%).



**Figure 9.** Temperature-dependent IR spectral variations in the 3050-2850  $\text{cm}^{-1}$  region of PHB and P(HB-*co*-HHx) (HHx=12 mol%). The spectra were measured over a temperature range of 20 °C to a higher temperature (PHB, 185 °C; HHx=12 mol%, 140 °C). (Reproduced from Ref. 23 with permission. Copyright (2004) American Chemical Society).

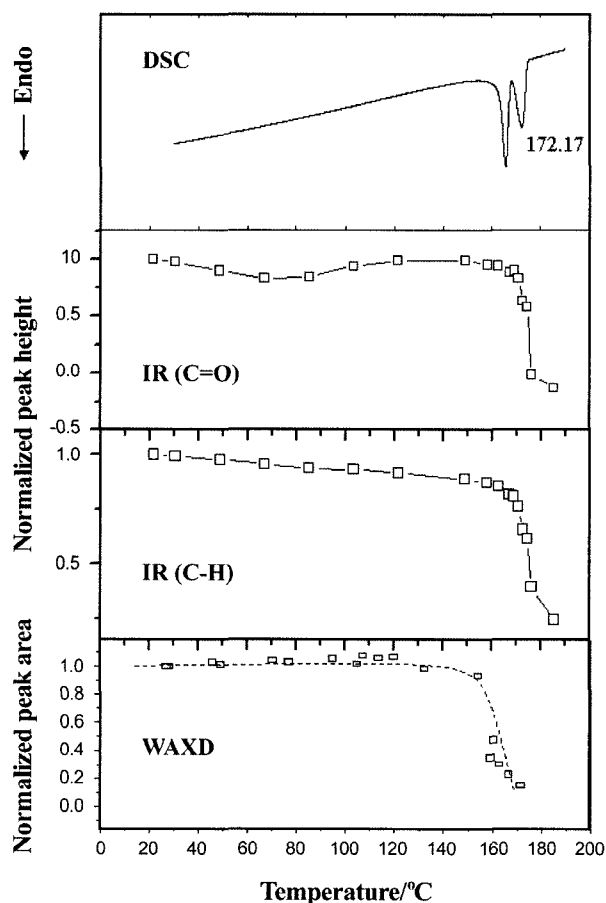
Figure 9 shows temperature-dependent spectral variations in the C-H stretching band region of PHB and P(HB-*co*-HHx) (HHx=12 mol%), respectively. It is noted that the  $\text{CH}_3$  asymmetric stretching band appears at 3009  $\text{cm}^{-1}$  for PHB and 3007  $\text{cm}^{-1}$  for P(HB-*co*-HHx) (HHx=12 mol%).<sup>23</sup> The appearance of the  $\text{CH}_3$  band can be detected more clearly in the second derivative spectra.<sup>23</sup> Recently, several research groups reported that the appearance of a  $\text{CH}_3$  asymmetric stretching band above 3000  $\text{cm}^{-1}$  indicates the possibility of a C-H $\cdots$ O=C hydrogen bond.<sup>27-29</sup> Therefore, the observation in Figure 9 together with the results of the temperature-dependent WAXD study suggest that the  $\text{CH}_3$  group in one helical structure and the C=O group in the other helical structure form the intermolecular C-H $\cdots$ O=C hydrogen bond in PHB and P(HB-*co*-HHx) (HHx=12 mol%).<sup>23</sup>

Figure 10 shows the DSC curve, the temperature dependences of the normalized peak heights of the C=O stretching band (crystalline band) at 1723  $\text{cm}^{-1}$  and the C-H stretching band at 3009  $\text{cm}^{-1}$ , and the normalized peak height of the (110)  $d$  spacing in the WAXD for PHB. It can be seen from Figure 10 that the crystalline structure of PHB deforms just below the melting temperature and that the C-H $\cdots$ O=C interaction is broken simultaneously.

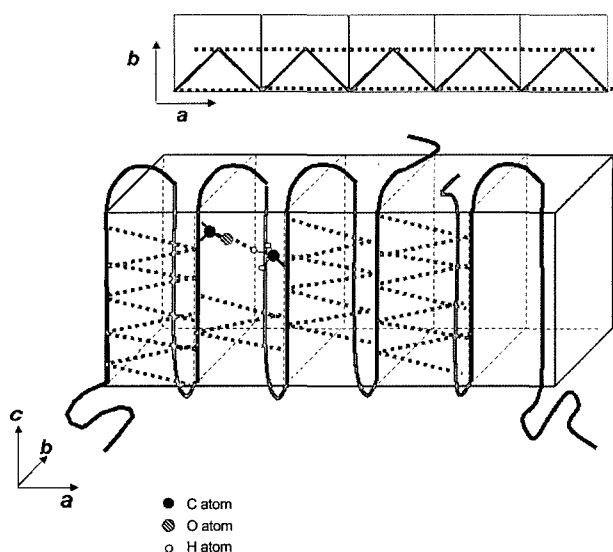
Figure 11 shows a proposed model for the lamellar structure of PHB and P(HB-*co*-HHx). The parallel helical structures are linked by a chain of C-H $\cdots$ O=C hydrogen bonds. It is very likely that the C-H $\cdots$ O=C hydrogen bonding stabilizes the chain folding in the lamellar structure of PHB and P(HB-*co*-HHx) and affects the difference in the thermal behavior between PHB and P(HB-*co*-HHx).

## Conclusions

The crystalline structure of PHB and of P(HB-*co*-HHx)



**Figure 10.** The DSC curve, normalized peak heights of the C=O stretching band at 1723  $\text{cm}^{-1}$  due to the crystalline state and the second derivative peak of the  $\text{CH}_3$  asymmetric stretching band at 3009  $\text{cm}^{-1}$ , and the (110) peak area of x-ray diffraction of PHB versus temperature.



**Figure 11.** A proposed model structure for the lamellae of PHB and P(HB-co-HHx).

has an orthorhombic system ( $\alpha=\beta=\gamma=90^\circ$ )  $P2_12_12_1(D_2^4)$ . The  $a$  and  $b$  lattice parameters increase with the increase in the HHx content because the propyl side chains of the HHx units expand the crystalline lattice. The temperature-dependent WAXD studies for PHB and P(HB-co-HHx) (HHx=2.5, 3.4, 10.5, and 12 mol%) reveal that the increasing ratios of the  $a$  and  $b$  lattice parameters are significantly different; the increasing ratio of the  $a$  lattice parameter is larger than that of the  $b$  lattice parameter. These observations suggest that there is an interaction along the  $a$  axis. It is very likely that the interaction is due to the C-H $\cdots$ O=C hydrogen bonding between the O atom of the C=O group and the H atom of the CH<sub>3</sub> group because they are located closely each other along the  $a$  axis. The distance between the O atom of the C=O group in one helical structure and the H atom of one of the three C-H bonds of the CH<sub>3</sub> group in the other helix structure was found to be 2.63 Å, which is shorter than the sum of van der Waals separation (2.72 Å). This result together with the appearance of the CH<sub>3</sub> asymmetric stretching band at 3009 cm<sup>-1</sup> lead us to conclude that there is a C-H $\cdots$ O=C hydrogen bond between the C=O and CH<sub>3</sub> groups in PHB and P(HB-co-HHx).

The temperature-dependent variations in the IR spectra for PHB and P(HB-co-HHx) (HHx=2.5, 3.4 mol%) revealed that they keep their crystalline structures until their melting points and that their helical structures are decomposed just below the melting temperature. However, the crystallinity and the helical structure of P(HB-co-HHx) (HHx=10.5 and 12 mol%) change gradually from fairly low temperature. The normalized peak height of the CH<sub>3</sub> asymmetric stretching band at 3009 cm<sup>-1</sup> of PHB and P(HB-co-HHx) shows similar thermal behavior to that of the C=O

stretching band at 1723 cm<sup>-1</sup> (Figure 10). These spectral changes are in good agreement with the results for the x-ray diffraction, DSC, and POM image. It is very likely that the C-H $\cdots$ O=C hydrogen bondings in PHB and its copolymers stabilize the lamellar structure, affecting the thermal behaviour, crystallinity, and stability of crystalline.

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## References

- (1) Y. Doi, *Microbial Polyesters*, VCH Publishers, New York, 1990.
- (2) M. Vert, *Biomacromolecules*, **6**, 538 (2005).
- (3) A. J. Anderson and E. Dawes, *Microbiol. Rev.*, **54**, 450 (1990).
- (4) E. A. Dawes, *Novel Biodegradable Microbial Polymers*, Dordrech Kluwer Academic, 1990.
- (5) L. M. Lara and W. H. Gjalt, *Microbiol. Mol. Biol. Rev.*, **63**, 21 (1991).
- (6) T. Iwata and Y. Doi, *Macromol. Chem. Phys.*, **200**, 2429 (1999).
- (7) P. J. Barham, P. Barker, and S. Organ, *FEMS Microbiol. Rev.*, **103**, 289 (1992).
- (8) P. A. Holmes, in *Developments in Crystalline Polymers*, D. C. Bassett, Ed., Elsevier, London, 1987, vol. 2, p 1.
- (9) N. Yoshie, M. Fujiwara, M. Ohmori, and Y. Inoue, *Polymer*, **42**, 8557 (2001).
- (10) R. H. Marchessault, S. Coulombe, H. Morikawa, K. Okamura, and J. F. Revol, *Can. J. Chem.*, **59**, 38 (1981).
- (11) E. Chiellini and R. Solaro, *Recent Advances in Biodegradable Polymers and Plastics*, Wiley-VCH, Weinheim, 2003.
- (12) Y. Doi, *ICBP 2003 First IUPAC International Conference on Bio-Based Polymers, Macromolecular Bioscience*, WILEY-VCH, Weinheim, 2004, vol. 4, Issue 3.
- (13) M. M. Satkowski, D. H. Melik, J.-P. Autran, P. R. Green, I. Noda, and L. A. Schechtman, in *Biopolymers*, A. Steinbüchel and Y. Doi, Eds., Wiley-VCH, Wienhiem, 2001, p 231.
- (14) M. Yokouchi, Y. Chatani, H. Tadokoro, K. Teranishi, and H. Tani, *Polymer*, **14**, 267 (1973).
- (15) J. Cornibert and R. H. Marchessault, *J. Mol. Biol.*, **71**, 735 (1972).
- (16) N. Yoshie, H. Menju, H. Sato, and Y. Inoue, *Macromolecules*, **28**, 6516 (1995).
- (17) Web site: www.nodax.com.
- (18) Y. Doi, S. Kitamura, and H. Abe, *Macromolecules*, **28**, 4822 (1995).
- (19) G. Kobayashi, T. Shiotani, Y. Shima, and Y. Doi, in *Biodegradable Plastics and Polymers*, Y. Doi and K. Fukuda, Eds., Elsevier, Amsterdam, 1994, p 410.

- (20) H. Abe, Y. Doi, H. Aoki, and T. Akehata, *Macromolecules*, **31**, 1791 (1998).
- (21) M. Kunioka, A. Tamaki, and Y. Doi, *Macromolecules*, **22**, 694 (1989).
- (22) H. Sato, M. Nakamura, A. Padermshoke, H. Yamaguchi, H. Terauchi, S. Ekgasit, I. Noda, and Y. Ozaki, *Macromolecules*, **37**, 3763 (2004).
- (23) H. Sato, R. Murakami, A. Padermshoke, F. Hirose, K. Senda, I. Noda, and Y. Ozaki, *Macromolecules*, **37**, 7203 (2004).
- (24) H. Sato, J. Dybal, R. Murakami, I. Noda, and Y. Ozaki, *J. Mol. Struct.*, **35-36**, 744 (2005).
- (25) H. Sato, A. Padermshoke, M. Nakamura, R. Murakami, F. Hirose, K. Senda, H. Terauchi, S. Ekgasit, I. Noda, and Y. Ozaki, *Macromol. Symp.*, **220**, 123 (2005).
- (26) R. H. Marchessault and J. Kawada, *Macromolecules*, **37**, 7418 (2004).
- (27) H. Matsuura, H. Yoshida, M. Hieda, S. Yamadaka, T. Harada, K. Shin-ya, and K. Ohno, *J. Am. Chem. Soc.*, **125**, 13910 (2003).
- (28) T. Harada, H. Yoshida, K. Ohno, and H. Matsuura, *Chem. Phys. Lett.*, **362**, 453 (2002).
- (29) P. Hobza and Z. Havlas, *Chem. Rev.*, **100**, 4253 (2000).