Melting behavior of poly(3-hydroxybutyrate) investigated by two-dimensional infrared correlation spectroscopy

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Received 31 March 2004; accepted 5 May 2004

Abstract

The melting behavior of a bacterially synthesized biodegradable polymer, poly(3-hydroxybutyrate) (PHB), was investigated by using generalized two-dimensional infrared (2D IR) correlation spectroscopy. Temperature-dependent spectral variations in the regions of the C–H stretching (3100–2850 cm⁻¹), C = O stretching (1800–1680 cm⁻¹), and C–O–C stretching (1320–1120 cm⁻¹) bands were monitored during the melting process. The asynchronous 2D correlation spectrum for the C = O stretching band region resolved two crystalline bands at 1731 and 1723 cm⁻¹. The intense band at 1723 cm⁻¹ may be due to the highly ordered crystalline part of PHB, and the weak band at 1731 cm⁻¹ possibly arises from the crystalline part with a less ordered structure. These crystalline bands at 1731 and 1723 cm⁻¹ share asynchronous cross peaks with a band at around 1740 cm⁻¹ assignable to the C = O band due to the amorphous component. This observation indicates that the decreases in the crystalline components do not proceed simultaneously with the increase in the amorphous component. In the 3020–2915 cm⁻¹ region where bands due to the asymmetric CH₃ stretching and antisymmetric CH₂ stretching modes are expected to appear, eight bands are identified at 3007, 2995, 2985, 2975, 2967, 2938, 2934, and 2929 cm⁻¹. The bands at 2985 and 2938 cm⁻¹ are ascribed to the amorphous part while the rest come from crystal field splitting, which is a characteristic of polymers with a helical structure.

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Keywords: Polyhydroxybutyrate; Melting behavior; 2D IR correlation spectroscopy

1. Introduction

Bacterially synthesized poly(3-hydroxyalkanoate)s (PHAs) are a class of naturally occurring biodegradable polyesters accumulated as energy-storing inclusion body granules in the cells of certain microorganisms [1–4]. Since PHAs can be biologically derived from renewable resources and are biodegradable, they have attracted a great attention as a new family of environmentally friendly polymeric materials [2,5–7]. Among various bacterially synthesized PHA polymers, poly(3-hydroxybutyrate) (PHB), the oldest known bacterial polyester, is the most abundant polyester found in bacteria [3,7]. The chemical structure of PHB, and its proposed helical structure [8,9] are shown in Fig. 3(A) and (B), respectively. PHB bears similar glass transition temperature ($T_g$), melting temperature ($T_m$), and comparable ultimate tensile strength to those of polypropylene (PP) [4,7]. PHB is perfectly biocompatible, and therefore its medical use is one of the important potential applications being considered. However, due to the high stereoregularity of biologically produced macromolecules, PHB is a highly crystalline polymer that is stiff and brittle. It is also thermally unstable during processing [7]. The molecular weight of PHB degrades significantly at temperature just above the $T_m$. This unfortunate aspect of properties poses a limitation of, for example, the application to a flexible film, which is one of the largest uses of biodegradable polymers. As a consequence, many attempts to copolymerize a comonomer with PHB monomer for improving its
mechanical properties have been made. One idea is to include a more bulky comonomer to reduce the crystallinity and presumably increase the flexibility of the resulting copolymers. The copolymerization with 3-hydroxyvalerate (3-HV) was the first attempt performed by ICI (Billingham, UK) in the early 1980s [7]. However, the crystallinity of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P(HB-co-HV)) never falls below 50% due to the isodimorphism of the P(HB-co-HV) copolymer [7]. It has been reported that poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(HB-co-HHx)) shows a greater drop in crystallinity, at a given mol% comonomer, in comparison to P(HB-co-HV) [7]. Interestingly, hexanoate and larger comonomers depress Tm in the same manner regardless of their molecular sizes [7]. This feature indicates the break down of the isodimorphism occurring in the P(HB-co-HV) copolymer by the incorporation of comonomer units with three or more carbon unit side groups [7].

The main factor to determine mechanical properties of the polymer is the degree of crystallinity. A fundamental understanding of the crystallization and melting process of this class of polymers is essential for improving their mechanical properties of biopolymers. We have been undertaking a series of studies on the structure and thermal behavior of PHB and P(HB-co-HHx), and the interactions decrease along the a axis of the crystal lattice of PHB and P(HB-co-HHx) with temperature [10,11]. IR spectroscopy has been successfully applied to the studies of crystalline/amorphous phase transition process of a number of polymers [12–15,17,18]. This is because IR absorption bands are very sensitive to changes in inter- and intramolecular interactions and conformational changes of polymers [17]. IR spectra of polymers are often complicated. In such a case, 2D correlation analysis proposed by Noda [18,19] enables a more detailed analysis of IR spectral variations of polymers [12–15,18–21]. The asynchronous 2D correlation spectrum generated for the C=O stretching band region of P(HB-co-HHx) revealed the coexistence of two crystalline bands at 1731 and 1723 cm\(^{-1}\) [12]. The band at 1723 cm\(^{-1}\) contributes to the highly ordered crystalline component, and that at 1731 cm\(^{-1}\) arises from the less ordered crystalline structure [12].

The present study aims at exploring the melting behavior of PHB by using 2D IR correlation spectroscopy. IR spectra of PHB, which is a basic polymer for all other PHA polymers, have never been analyzed in detail. Therefore, the outcome of the present study is important not only for the study of PHB itself but also for the future study of other PHAs. In order to extract as much information related with the phase transition behavior of PHB polymer as possible, we analyzed not only the C=O stretching band region but also the C–O–C stretching band and C–H stretching band regions by employing 2D IR correlation spectroscopy. 2D IR correlation spectroscopy was successfully employed for studying the phase transitions of a number of PHA copolymers [12–15].

2. Experimental

2.1. Materials

The purified PHB sample was provided by the Procter and Gamble Company (Cincinnati, USA) and used as received. Chloroform (CHCl\(_3\)) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and used as a solvent without further purification.

2.2. IR spectroscopic measurements

A film of PHB sample was prepared by casting its chloroform solution on a CaF\(_2\) window. The film was kept in a vacuum-dried oven at 60 °C for 12 h and cooled down to room temperature. IR spectra of the PHB film were collected at a 2 cm\(^{-1}\) spectral resolution by using a Nicolet Magna-IR\(_{\text{TM}}\) 560 spectrometer equipped with a mercury cadmium telluride (MCT) detector. To ensure a high signal-to-noise ratio, 512 scans were co-added. The temperature of the sample cell that holds the CaF\(_2\) window with
the PHB film was controlled by using a temperature controller unit (CHINO, model SU). The spectral acquisitions were performed over a temperature range of 40–180 °C with an increment of 10 °C.

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 IR spectra were used as the reference spectra for the 2D correlation calculations.

3. Results and discussion

3.1. Temperature-dependent changes in the C=O stretching vibration region

Fig. 2 shows temperature-dependent IR spectra of PHB in the 1800–1680 cm⁻¹ region where bands due to the C=O stretching modes are expected to appear. The band shape in the C=O stretching vibration region changes largely from a sharp peak with a broad shoulder to a symmetrical broad band with increasing temperature. A dramatic change in the IR spectra was observed in the vicinity of 140 °C. PHB is known as a semi-crystalline polymer, and the Tm of PHB was reported to be 170 °C [11]. The spectral changes observed in Fig. 2 reflect the melting of PHB. The C=O stretching band region consists of at least two dominant bands. The sharp band at 1723 cm⁻¹ arises from the crystalline C=O groups, and the broad band at 1740 cm⁻¹ is attributed to the amorphous C=O groups [12–16]. The band at 1723 cm⁻¹ decreases with increasing temperature and disappears above the Tm while the band at 1740 cm⁻¹ gradually increases. Accordingly, the band at 1723 cm⁻¹ is a unique indicator for monitoring the melting or crystallization process of PHA polymers.

There are two possible interpretations for the origin of the splitting of the C=O band observed below the Tm: (1) an interaction between the C=O and CH₃ groups [10,11,22] and (2) a dipole-dipole coupling interaction between ester groups in an ordered structure [23–25]. It was reported that PHB assumes an orthorhombic crystal system, P2₁2₁2₁(D₂h), (a = b = γ = 90°) with a = 5.76 Å, b = 13.20 Å, c = 5.96 Å (fiber axis) [9,26]. The x-ray crystallographic studies of PHB showed that the distance between the C=O and CH₃ groups along the a axis is close enough for supposing the interactions between them. We studied the thermal properties of PHB by using WAXD and DSC [10,11]. The temperature-dependent variations in WAXD of PHB revealed that the lattice parameter a shows a significant variation with the temperature increase. In other words, the thermal energy weakens the interaction along the a axis. The change in the interaction between the C=O and CH₃ groups may cause the change in the C=O stretching band. The sharp peak at 1723 cm⁻¹ may arise from the C=O groups involved in the C=O and CH₃ interactions while the broad feature at 1740 cm⁻¹ may be due to the free or non-interaction C=O groups.

Another possibility that causes the C=O band splitting is the transition dipole coupling of the ester groups in an ordered structure [23–25]. For methyl acetate, which can be a model compound for the ester group incorporated in the main chain of PHB, the dipole practically lies along the C=O bond. Galbiati et al. [25] measured IR and Raman spectra of several aliphatic esters in gas, liquid, and solid phases. It was revealed that the observed frequencies of the C=O stretching vibrations (νC=O) of the compounds can be grouped into three wavenumber regions for the three phases: (1) νC=O > 1760 cm⁻¹ for the gas phase, (2) 1750 cm⁻¹ > νC=O > 1740 cm⁻¹ for the liquid phase, and (3) 1730 cm⁻¹ > νC=O > 1720 cm⁻¹ for the solid phase. They also performed MNDO (modified neglect of differential overlap) semiempirical quantum chemical calculation for estimating the minimum-energy geometry and dipole moment and derived the following results: the dipole-dipole interaction causes a lower frequency shift of the C=O stretching band, and the maximum distance capable of producing a band splitting is not larger than ca. 4–5 Å [25]. In polymer systems, a band splitting due to the dipole-dipole interaction (often called dipole-dipole coupling) is observed when the molecules form an ordered structure [24,27,28]. Tori and Tatsu showed that the amide I bands of several proteins, which contain the ordered structures such as α-helices and β-sheet, are successfully reproduced by the model calculation based on the dipole-dipole coupling mechanism [27,28].
Referring to the stereograph of the molecular structure for PHB in a crystalline state determined by X-ray diffraction [9], the polymer chains form a helical structure, and the crystal lattice of the polymer contains two left-handed helical molecules in an antiparallel orientation. In the helical structure, the distance between the neighboring C=O groups along the chain is ca. 4–5 Å. Thus, the splitting of the C=O stretching band of PHB observed below the $T_m$ possibly arises from the dipole-dipole interaction between the C=O groups in the crystalline structure.
Fig. 4. A second derivative spectrum calculated from the infrared spectrum of PHB in the C=O stretching vibration region at 40 °C.

To extract more detailed information about the spectral variations taking place during the phase transition process, 2D correlation spectroscopy was employed. Fig. 3(A) and (B) shows the synchronous and asynchronous 2D correlation spectra, respectively, in the spectral region of 1780–1700 cm\(^{-1}\) generated from the temperature-dependent IR spectra measured over a temperature range of 40–150 °C. Two autopeaks developed at around 1744 and 1724 cm\(^{-1}\) together with negative cross peaks at ∼(1744, 1724) cm\(^{-1}\) indicate the spectral variations of the amorphous and crystalline components of the PHB polymer. Three pairs of cross peaks are observed at ∼(1744, 1731), ∼(1744, 1722), and ∼(1731, 1722) cm\(^{-1}\) in the corresponding asynchronous spectrum. It should be noted that the existences of these bands are confirmed also by the second derivative of the spectrum of PHB at 40 °C shown in Fig. 4. Although the band at 1731 cm\(^{-1}\) presents as a small peak in the second derivative spectrum, the asynchronous 2D spectrum clearly sorts out the existence of this band. The second derivative spectrum depicts two bands at 1748 and 1723 cm\(^{-1}\) in the amorphous region. These two bands, however, show no asynchronicity with each other in the 2D asynchronous map. The positive synchronous spectral intensity in the region containing the coordinate at ∼(1731, 1722) cm\(^{-1}\) indicates the same direction of the intensity variations of these two bands while the negative synchronous spectral intensity containing the coordinates at ∼(1744, 1731) and ∼(1744, 1722) cm\(^{-1}\) suggests that the broad band located around 1744 cm\(^{-1}\) varies in the opposite direction from the two bands at 1731 and 1722 cm\(^{-1}\). The highly overlapped bands located around 1744 and 1722 cm\(^{-1}\) are not resolved in the synchronous map because, unlike an asynchronous spectrum, which is powerful in differentiating bands arising from different origins, a synchronous spectrum generally shows overall similarities of spectral variations. The frequencies of the bands in the C=O stretching vibration region and their assignments are summarized in Table 1.

The asynchronous 2D correlation spectrum resolves the two highly overlapped bands at around 1731 and 1722 cm\(^{-1}\) arising from the crystalline state. It can be seen from Fig. 4 that the band at 1722 cm\(^{-1}\) is much stronger than that at 1731 cm\(^{-1}\). Based on its frequency and intensity, the band at 1722 cm\(^{-1}\) may be assigned to the C=O stretching mode of the well-ordered crystalline state of PHB [12]. The minor

Table 1

<table>
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<tr>
<th>Functional groups</th>
<th>Wavenumber (cm(^{-1}))</th>
<th>Assignments</th>
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<tr>
<td></td>
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<td>crystalline</td>
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<tr>
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<tr>
<td></td>
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</table>

Fig. 5. IR spectra of PHB in the C–O–C stretching vibration region (1320–1120 cm\(^{-1}\)) collected over a temperature range of 40–180 °C.
band at 1731 cm\(^{-1}\) may be due to the C=O stretching mode of the less ordered crystalline part of the polymer [12].

Of note in the asynchronous map is that there are asynchronous cross peaks between the crystalline and amorphous bands. This indicates that the decreases in the crystalline components and the increase in the amorphous component take place out of phase. It is very likely that the melting of PHB occurs through an intermediate state. However, no
IR band due to the intermediate state is clearly detected. In our previous study [12], it was revealed that the melting of P(HB-co-HHx) (12 mol% HHx) proceeds through an intermediate state. Wu et al. [13] and Tian et al. [14] investigated the melting behavior of P(HB-co-HHx) (10 mol% HHx) and P(HB-co-HV) (20.4 mol% HV), respectively, by using 2D IR correlation spectroscopy and suggested from the asynchronous spectra for the C=O stretching region that an intermediate state appears in the course of the melting process. Thus, the present study indicates that an intermediate state exists commonly in the melting process of this class of polymers.

3.2 Temperature-dependent changes in the C–O–C stretching vibration region

Fig. 5 shows the temperature-dependent changes in the IR spectra of PHB in the 1320–1120 cm$^{-1}$ region. The cluster of bands in this region is ascribed to the stretching vibrations of the C–O–C groups [12–15]. Although this region contains more bands than the C=O stretching vibration region, the spectral changes caused by the melting process of the polymer are clear. The intensities of the bands at 1294, 1280, 1263, and 1230 cm$^{-1}$ decrease upon passing from the crystalline to the amorphous state of the polymer, whereas those of the bands at 1303, 1259, and 1183 cm$^{-1}$ increase in the course of temperature increase.

The synchronous and asynchronous 2D correlation spectra in the 1330–1155 cm$^{-1}$ region calculated from the temperature-dependent IR spectra of PHB are shown in Fig. 6(A) and (B), respectively. There are at least four autopeaks at 1294, 1254, 1230, and 1180 cm$^{-1}$. The autopeak at 1294 cm$^{-1}$ extends to the vicinity of 1280 cm$^{-1}$, and that near 1180 cm$^{-1}$ is very broad. Thus, it seems that both autopeaks consist of more than one peak. A pair of asynchronous peaks at (1188, 1170 cm$^{-1}$) may be due to a band shift, but the autopeak near 1180 cm$^{-1}$ is so broad and thus, there may be two bands near 1180 cm$^{-1}$. It is noted that the crystalline bands at 1294, 1280, 1263, and 1230 cm$^{-1}$ do not share any asynchronous peak, and that there are several cross peaks between an amorphous band (e.g., 1180 cm$^{-1}$) and crystalline bands (e.g., 1294–1280 and 1230 cm$^{-1}$). These observations again indicate the existence of an intermediate state during the phase transition. The frequencies of the bands in the C–O–C stretching vibration region and their assignments are summarized in Table 1.

3.3 Temperature-dependent changes in the C–H stretching vibration region

The temperature-dependent changes in the IR spectra of PHB in the 3100–2850 cm$^{-1}$ region are shown in Fig. 7. For the C–H stretching vibration region, bands located around 2980 cm$^{-1}$ are due to the CH$_3$ asymmetric stretching mode while those appeared around 2930 cm$^{-1}$ are attributed to the CH$_3$ antisymmetric stretching vibration. For PHB, the CH$_2$ group exists only on the side chain while the CH$_3$ group is located on the skeletal chain. As described in the introduction, the chemical modification on the side chain of PHB is very important for improving its mechanical properties, therefore the investigation on the thermal behavior of the CH$_3$ group is very interesting.

As temperature increases, the CH$_3$ stretching band at 2975 cm$^{-1}$ decreases while that at 2985 cm$^{-1}$ increases. Similarly, the CH$_2$ stretching band at 2934 cm$^{-1}$ decreases while that at 2938 cm$^{-1}$ increases with increasing...
Fig. 9. Synchronous (A) and asynchronous (B) correlation spectra of PHB in the C–H stretching vibration region constructed from dynamic IR spectra in the melting process.

temperature. Note that at room temperature, there exist two peaks at 3007 and 2995 cm$^{-1}$, but they disappear at 180 °C. These two bands and the band at 2975 cm$^{-1}$ may be ascribed to the crystalline CH$_3$ stretching vibration bands resulting from the crystal field splitting of the band at 2985 cm$^{-1}$. Crystal field splittings of bands occur in some semi-crystalline polymers [17]. The crystal field splitting may be caused not only by the intramolecular interaction between analogous functional groups attached along a polymer chain but also by the intermolecular interaction be-
Fig. 10. Hetero-correlation synchronous spectrum of PHB between the C=O and C–H stretching vibration regions constructed from dynamic IR spectra in the melting process.

tween those groups located in the crystalline lattice. The crystalline band splittings of certain vibration modes have been reported for a number of polymers especially those having a helical chain structure [17]. The second derivatives of the IR spectra of PHB in this region for the amorphous (at 180 °C) and crystalline (at 40 °C) states are shown in Fig. 8. Two additional bands at 2967 and 2929 cm⁻¹ are detected in the second derivative spectrum for the crystalline state.

Fig. 9(A) and (B) illustrates the synchronous and asynchronous 2D correlation spectra in the 3020–2915 cm⁻¹ region constructed from the temperature-dependent IR spectra. Four autopeaks are clearly observed at 3009, 2985, 2975, and 2930 cm⁻¹ in the synchronous map. The three peaks at 3009, 2975, and 2930 cm⁻¹ share three positive cross peaks at (3009, 2975), (3009, 2930), and (2975, 2930) cm⁻¹, and the peak at 2985 cm⁻¹ shares negative cross peaks with those at 3009, 2975, and 2930 cm⁻¹. These observations confirm that the three bands at 3009, 2975, and 2934 cm⁻¹ vary in the same direction, which is opposite to that of the band at 2985 cm⁻¹, with the change in temperature.

The asynchronous map and the second derivatives (Fig. 8) reveal that there are three bands at ~2940, 2934, and 2929 cm⁻¹ assignable to the antisymmetric CH₂ stretching mode. The amorphous band at 2985 cm⁻¹ shares asynchronous cross peaks with the crystalline bands at 3009, 2975, 2934, and 2929 cm⁻¹. This also suggests that the amorphous phase does not appear simultaneously with the disappearance of the crystalline state. The synchronous and asynchronous 2D correlation spectra in the C–H stretching vibration region reveal clearly that the intensity changes of all crystalline bands in this region occur simultaneously, i.e., there is no asynchronicity between any crystalline band in the C–H stretching vibration region. Therefore, it is very likely that the crystalline bands observed in this region are caused by the crystal field splittings.

A synchronous 2D correlation spectrum between the C=O and C–H stretching regions was generated from the temperature-dependent IR spectra and is demonstrated in Fig. 10. The synchronous cross peaks and their signs at the spectral coordinates of 1745 and 1725 cm⁻¹ versus 3009, 2986, 2975, and 2930 cm⁻¹ clarify that the bands located at 3007, 2975, 2934, and 2929 cm⁻¹ arise from the crystalline part of the polymer while the band at 2985 cm⁻¹ is ascribed to the amorphous part. The frequencies of the bands in the C–H stretching vibration region and their assignments are summarized in Table 1.

We also measured the temperature-dependent IR spectra of PHB during a cooling down process. 2D correlation maps for the C=O and C–H stretching regions was generated from the temperature-dependent IR spectra and is demonstrated in Fig. 10. The synchronous cross peaks and their signs at the spectral coordinates of 1745 and 1725 cm⁻¹ versus 3009, 2986, 2975, and 2930 cm⁻¹ clarify that the bands located at 3007, 2975, 2934, and 2929 cm⁻¹ arise from the crystalline part of the polymer while the band at 2985 cm⁻¹ is ascribed to the amorphous part. The frequencies of the bands in the C–H stretching vibration region and their assignments are summarized in Table 1.

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4. Conclusion

The melting behavior of PHB was investigated by using generalized 2D IR correlation spectroscopy. The spectral intensities in the C=O, C–O–C, and C–H stretching vibration regions are markedly changed with the temperature change. The crystalline bands decrease while the amorphous bands increase during the melting process. This is because the highly ordered and helical structure of the PHB polymer is diminished at high temperature.

The asynchronous 2D correlation spectrum generated from the temperature-induced dynamic IR spectra in the C=O stretching vibration region resolves two highly overlapped crystalline bands located at around 1731 and 1722 cm\(^{-1}\). The coexistence of these two bands is confirmed also by the second derivative of the spectrum of PHB in the C=O stretching band region at 40\(^{\circ}\)C. The intense band at 1722 cm\(^{-1}\) may be attributed to the major crystalline part of the polymer, and the much weaker feature at around 1731 cm\(^{-1}\) is possibly assignable to the minor crystalline component with a less ordered structure. The appearances of the asynchronous cross peaks at \((1744, 1731)\) and \((1744, 1722)\) cm\(^{-1}\) indicate the out-of-phase variations of the crystalline and amorphous parts, suggesting the existence of an intermediate state in the melting process.

Bands in the C–O–C and C–H stretching regions have also been classified into those arising from the crystalline and amorphous parts. The CH\(_3\) asymmetric stretching bands and CH\(_2\) antisymmetric stretching bands of the crystalline phase split into four bands at 3007, 2995, 2975, and 2967 cm\(^{-1}\), and two bands at 2934 and 2929 cm\(^{-1}\), respectively. This splitting is very likely due to crystal field splitting, which is often observed for polymers with a helical structure. These bands share positive synchronous cross peaks, and there is no asynchronic feature among them, confirming that these bands arise from the crystal filed splitting.

Acknowledgements

A.P. gratefully thanks the Thailand Research Fund (TRF) for the financial support via the Royal Golden Jubilee (RGJ) Ph.D. program (Grant contract number: PHD/0066/2544).

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