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Structure investigation of narrow banded spherulites in polyhydroxyalkanoates by microbeam X-ray diffraction with synchrotron radiation

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Abstract

Narrow banded structures of spherulites of poly[(*R*)-3-hydroxybutyrate] (P(3HB)) and its copolymers, with different second monomer unit of 4-hydroxybutyrate, 3-hydroxyhexanoate or 6-hydroxyhexanoate, were investigated by microbeam X-ray diffraction with Fresnel Zone Plate technique in synchrotron radiation (SPring-8). Radial scanning of spherulites were performed in 2 or 4 μm steps with 0.5 μm beam diameter of fine microfocus beam. The X-ray diffraction patterns for P(3HB) homopolymer spherulites showed the change of crystal orientation along the radial direction (*a*-axis), while the *b*- and *c*-axes rotated around *a*-axis. The intensities in microbeam X-ray diagrams of spherulites for P(3HB) copolymers changed periodically as function of the distance along the radial direction. The periodicity measured by X-ray diffraction was matched with narrow band spacing (15–25 μm) observed by polarized optical microscopy.

Keywords: Biodegradable polymer; Poly[(*R*)-3-hydroxybutyrate] copolymers; Banded spherulites

1. Introduction

Poly[(*R*)-3-hydroxybutyrate] (P(3HB)) and its copolymers are a family of chiral aliphatic polyesters synthesized from renewable carbon sources by a number of bacteria [1–3]. These microbial polyesters have attracted much attention because of their biodegradability and biocompatibility [1].

The melt-crystallized films of P(3HB) [4–6] and its copolymers [7–12] show the typical concentric alternating extinction banded spherulites, in the cross-polarized optical microscope. Generally, the band spacing increases with crystallization temperature, and the spherulitic morphology and degree of crystallinity are dependent on the crystallization conditions [4,13]. Many researchers have attempted to investigate the morphology of spherulites and crystallization mechanism of lamellar structure [1,4–23]. The extinction bands were attributed to the helicoidal twist of radial lamellar ribbons. The phenomena causing such banded spherulites were interpreted by the twisted crystal model [14,15]. Several fundamentally different models have been proposed to explain the driving force for twisting [16–23]. However, the mechanism on twisting during crystallization or growth direction of lamellar crystals is still unclear.

Atomic force microscopy (AFM) has been widely used to image the surface topography on a nanometer scale and to measure the chemical, adhesive, and elastic properties. Recently, several investigations for poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HH)) banded spherulites and terraces have directly observed a crystal twisting by atomic force microscopy [24–26].

Microbeam X-ray diffraction is a useful and powerful method to investigate the transition of crystalline region and local structure for monofilament [27,28]. A complete P(3HB) spherulite has recently been mapped by means of microbeam X-ray diffraction using a synchrotron source

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with 7 μm beam [29], and the radial orientation of the *a*-axis for spherulites was reported. Structural variations within P(3HB) banded spherulites (∼20–25 μm thickness) blended with the non-crystallizable atactic-P(3HB) (*a*-P(3HB)) have been achieved by using a practically two-dimensional spherulites with wide extinction bands (band spacing >80 μm) and a 3 μm beam [30]. The results clearly showed that the observed structural changes were directly correlated with the morphological features responsible for banding. The wide banded structure of P(3HB) spherulites (∼30 μm thickness and 120 μm band spacing) were also investigated by using a 3 μm beam [31]. The unit cell smoothly rotated around the *a*-axis with increasing distance from the spherulite center. Furthermore, poly(*L*-lactic acid) (PLLA) spherulites (∼20–25 μm thickness) blended with *a*-P(3HB) have been investigated with 3 μm beam diameter [32]. However, the spherulites with narrow band spacing (typically 10 μm) such as P(3HB) copolymers have not been investigated with high-resolution by limiting of beam size.

In the present paper, wide-angle microbeam X-ray diffraction using synchrotron radiation with 0.5 μm beam size was used to investigate the spherulites of P(3HB) and its copolymers with narrow band spacing (∼10–30 μm interval) in thick samples (∼50–100 μm thickness). The aim of this work was to gain the diffraction patterns about structural variation in concentric alternating band and the determination of orientation along the radial direction.

2. Experimental

2.1. Materials

Poly[(*R*)-3-hydroxybutyrate] (P(3HB)), poly[(*R*)-3-hydroxybutyrate-*co*-8 mol%-4-hydroxybutyrate] (P(3HB-*co*-8%-4HB)), and poly[(*R*)-3-hydroxybutyrate-*co*-8 mol%-(*R*)-3-hydroxyhexanoate] (P(3HB-*co*-8%-3HH)), were prepared by the microbial synthetic methods [8,33–35]. Poly[(*R*)-3-hydroxybutyrate-*co*-10 mol%-6-hydroxyhexanoate] (P(3HB-*co*-10%-6HH)) was synthesized by the ring-opening copolymerization of (*R*)-β-butyrolactone (ee 92%) with ε-caprolactone in the presence of tin-based catalyst [9]. Number-average molecular weight (M_n), and polydispersity (M_w/M_n) of P(3HB) homopolymer and its copolymers were summarized in Table 1. By analysis of the ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra of copolyesters, the sequence distributions of (*R*)-3HB and other hydroxyalkanoic acid units in all copolyesters were found to be statistically random [7–9,36].

2.2. Preparation of spherulites from melt-crystallized film

Films of P(3HB) and three kind of P(3HB) copolymers were initially prepared by solvent-cast technique from chloroform solutions of polymers. The solvent-cast films on the glass slide were heated on the hot-press. Samples were melted at 20–30 °C higher temperature than melting temperature (T_m) for 30 s, and then were crystallized isothermally at a given crystallization temperature (T_c) in hot oven. Crystallization conditions and film thickness were summarized in Table 1. Spherulitic morphologies of melt-crystallized films were observed with an optical microscope (Nikon Eclipse-E600POL) equipped with cross polarizer. Spherulite size and band spacing were measured after calibration with a micrometric graticule.

2.3. Wide-angle microbeam X-ray diffraction

The microbeam X-ray diffraction was carried out at beam line BL47XU with wavelength of 0.15497 nm at 8 keV of synchrotron radiation at SPring-8, Harima, Japan. The total flux of microbeam is about 10^9 photons/s for 100 mA stored current at a X-ray energy of 8 keV. The beam size was obtained as 0.5 μm by using Fresnel Zone Plate [37].

The isothermally crystallized films (30–110 μm thickness) were recovered from slide glass, and cut about 10 mm in size. The surface of the samples was set perpendicular to the X-ray beam and therefore parallel to the detector. The region for scanning of spherulites was monitored in video microscope. The center position of X-ray beam was adjusted to the desired position in spherulites according to the method reported previously [37]. For each sample, a linear scan with 2 or 4 μm steps was performed along the spherulite radius. The diffraction patterns were recorded with a CCD camera (C4880-10-14A, Hamamatsu Photonics, Japan) with exposure time of 5–20 s. The camera length was 70 mm. The fiber pattern simulation of P(3HB) homopolymer was generated with Cerius^2 software package [38] by using the atomic coordinates of P(3HB) molecules [39].

Table 1
Molecular weights, film thickness, crystallization temperature to prepare spherulites and morphological parameters of P(3HB) and its copolymer samples

Sample	$M_n \times 10^{-3}$	M_w/M_n	T_c (°C)	Thickness (μm)	Average band spacing[a] (μm)	Periodicity[b] (μm)
P(3HB)	55	2.0	140	30	70	100
P(3HB-*co*-8%-4HB)	197	1.9	120	50	15	12
P(3HB-*co*-8%-3HH)	279	2.4	110	110	20	20
P(3HB-*co*-10%-6HH)	107	1.7	100	90	25	20

[a] Measured by optical microscopy.

[b] Measured by X-ray diffraction.

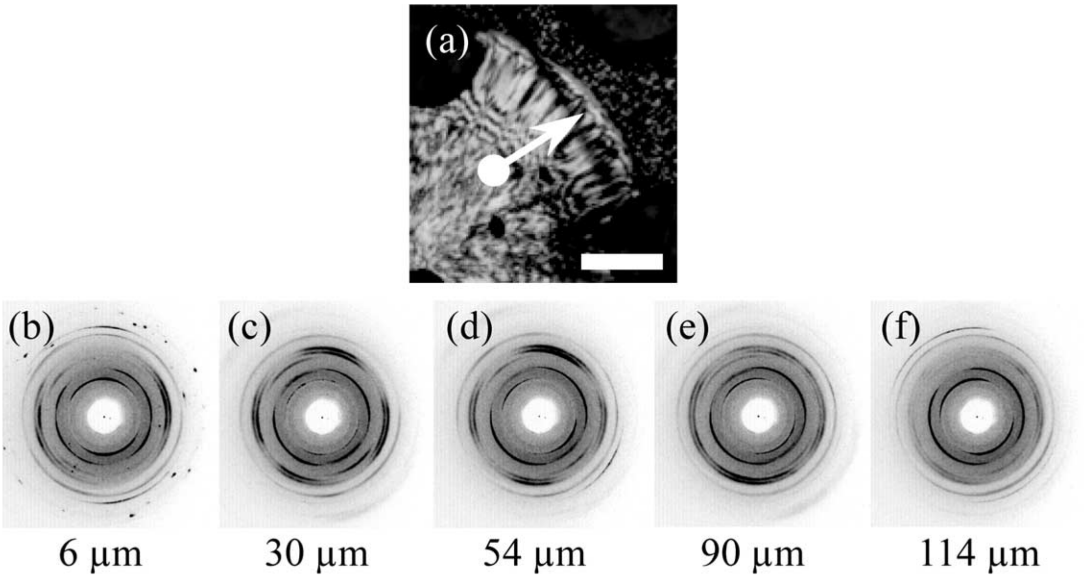

Fig. 1. (a) Polarized optical micrograph of P(3HB) spherulite, investigated by microbeam X-ray diffraction. The arrow shows the position where the scan was performed. The scale bar is 200 μm. The X-ray diffraction patterns were recorded from the spherulite center to outward; (b) 6 μm, (c) 30 μm, (d) 54 μm, (e) 90 μm, and (f) 114 μm.

3. Results and discussion

3.1. P(3HB) spherulite

Fig. 1(a) shows the optical micrograph of P(3HB) spherulite in thin film (ca. 30 μm thickness). P(3HB) spherulite with band spacing of 70 μm was investigated by 0.5 μm microbeam X-ray diffraction along the radial direction. A 150 μm segment was scanned in 3 μm steps toward the direction indicated by arrow in Fig. 1(a). The selected X-ray diffraction patterns obtained at 6, 30, 54, 90, and 114 μm point from spherulite center are shown in Fig. 1(b)–(f), respectively. While the regularity of crystal orientation in spherulites was slightly lack, X-ray diffraction patterns of P(3HB) spherulite changed dramatically as function of the distance from its center. The X-ray diffraction pattern (Fig. 1(f)) at 114 μm point from starting was the almost same with that at 6 μm point (Fig. 1(b)). This agreement indicates that the structural orientation of P(3HB) varied along the radial direction with the interval

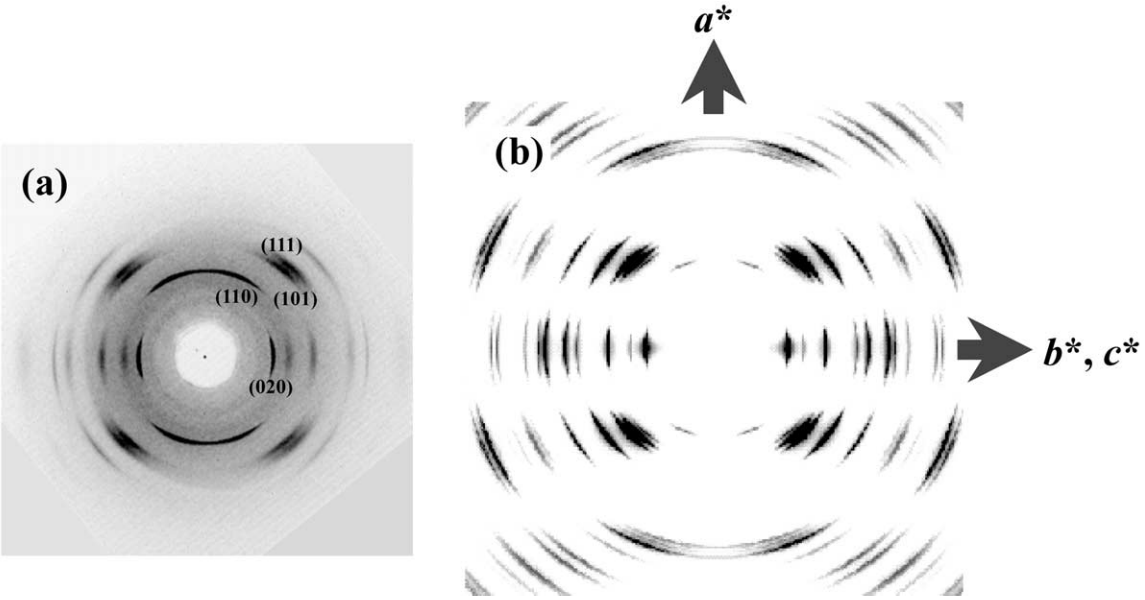

Fig. 2. (a) Microbeam X-ray diffraction pattern of P(3HB) spherulite at the distance of 150 μm from the center, and (b) a simulated diffraction pattern of P(3HB) in α-crystal form by Cerius^2.

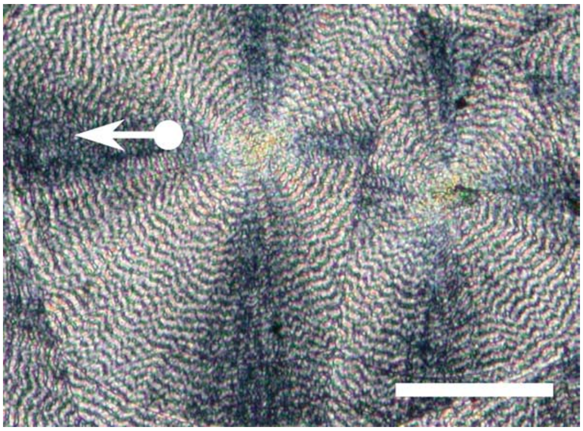

Fig. 3. Polarized optical micrograph of P(3HB-*co*-8%-4HB) spherulites investigated by microbeam X-ray diffraction. The arrow shows the position where the scanning was performed. The scale bar is 200 μm.

of ca. 100 μm in this spherulite, which is close to the approximate value with the band spacing of ca. 70 μm measured by optical microscopy.

The X-ray diffraction pattern at 150 μm point from starting in spherulites is shown in Fig. 2(a). Fig. 2(b) shows a simulated pattern by using the known crystal parameters of the α-form of P(3HB) ($a=0.576$ nm, $b=1.320$ nm, and c (fiber axis) $=0.596$ nm and space group $P2_12_12_1$ [39]) and assuming a fiber pattern where the crystallographic a-axis is the uniaxial direction and the b- and c-axes are oriented randomly perpendicular to a-axis. The very good agreement between the simulated (Fig. 2(b)) and observed (Fig. 2(a)) patterns suggests that P(3HB) crystallizes in the α-form and that the crystalline unit cells in spherulites are oriented with the a-axis along the radial direction. Therefore, these results make a clearly show that the unit cell rotates around a-axis with the constant period from the spherulites center. This result is supported by the oscillation changes of X-ray patterns and of intensities in the reflections (020) and (002) for bacterial-P(3HB) homopolymer using a 3 μm beam [32].

3.2. P(3HB) copolymer spherulites

It is well known that spherulites of P(3HB) copolymers have narrow band spacing of typically ca. 10–20 μm [8–12]. Until now, narrow banded spherulites could not allow high-resolution structural analysis. In this study, a fine microbeam of 0.5 μm by using Fresnel Zone Plate [37] can use first to analyze for the structure of narrow banded spherulites of ca. 15–25 μm. In this study, measurements were performed against thick samples with ∼50–100 μm thickness.

Fig. 3 shows the optical micrograph of P(3HB-*co*-8%-4HB) spherulites (ca. 50 μm thickness) that have maltese cross and narrow banding of ca. 15 μm spacing. A 40 μm distance was scanned in 2 μm steps toward the direction indicated by the arrow in Fig. 3. The selected X-ray diffraction patterns for spherulites of P(3HB-*co*-8%-4HB) obtained at 2, 4, 6, 8, 10, and 12 μm point from spherulite center are shown in Fig. 4(a)–(f), respectively. The intensities of reflections were different dependent on the

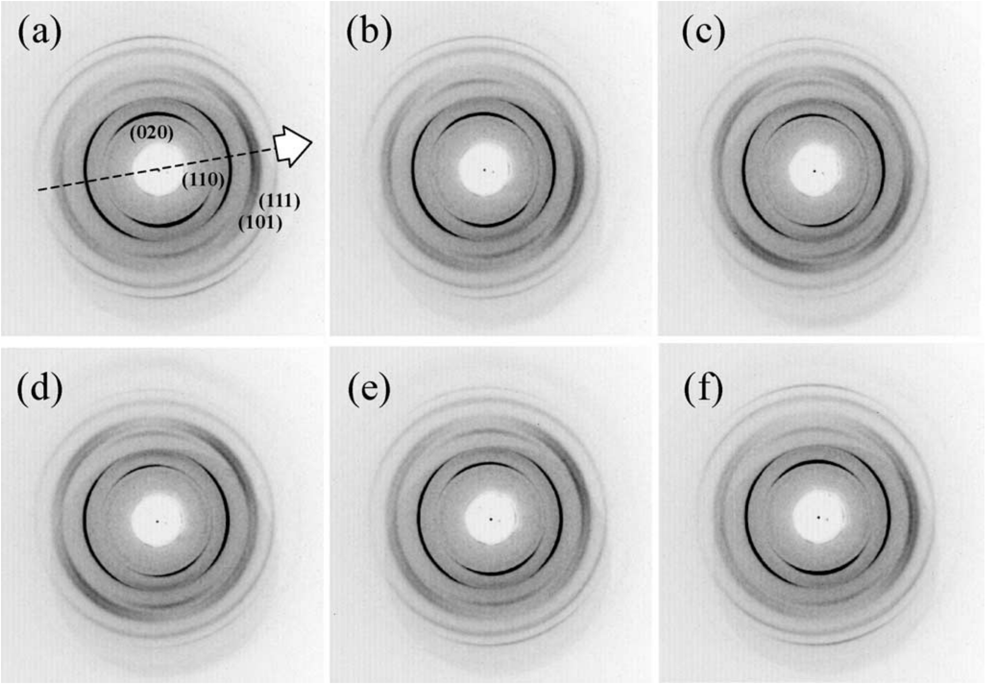

Fig. 4. Microbeam X-ray diffraction patterns of P(3HB-*co*-8%-4HB) spherulite along spherulite radius. The patterns were recorded from the spherulite center to outward: (a) 2 μm, (b) 4 μm, (c) 6 μm, (d) 8 μm, (e) 10 μm, and (f) 12 μm. The white arrow indicates the growth direction of spherulite defined as 0° for azimuthal angle, respectively.

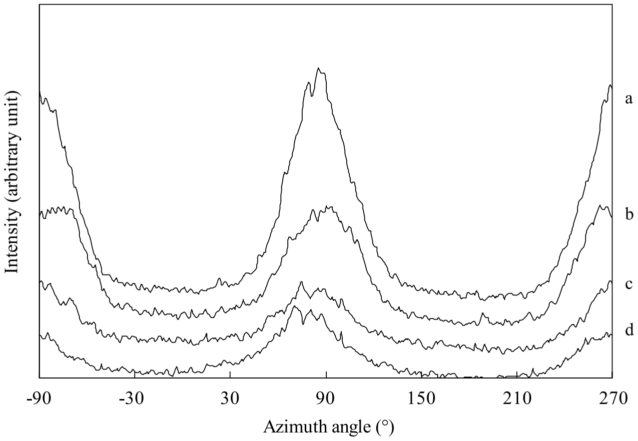

Fig. 5. Azimuthal distribution of the intensity of (020) reflection (marked by the black arrow in Fig. 4(a)) of P(3HB-*co*-8%-4HB) spherulite; (a) 2 μm, (b) 4 μm, (c) 6 μm, and (d) 8 μm.

distance from center. In particular, it is noted that the (020) reflection arrowed in Fig. 4(a) remarkably changed. Azimuthal distributions of intensity for (020) reflection in Fig. 4(a)–(d) are shown in Fig. 5. When the growth direction of spherulite was defined as 0°, one can observe two peaks at 90 and 270°. Two intensities at 90 and 270° for azimuthal angle decreased with increasing in the distance from 2 to 8 μm. Fig. 6 shows the changes of intensities at 90 and 270° for azimuthal angle corresponding to (020) reflection in all measured range. The trend of variation at 90° for azimuthal angle was same as that at 270° for all range. Clearly, there are the periodic changes for intensity of X-ray diffraction as function of the scanning distance from 0 to 40 μm. The period obtained from the change of intensity was ca. 12 μm. This value was good agreement with band spacing of ca. 15 μm observed by polarized optical microscopy. Therefore, the oscillation period obtained from the change of intensities for azimuthal angle in (020) reflection by using microbeam with 0.5 μm shows periodically structural variation, even if the band spacing is very narrow and sample thickness is thick. The rotation of lamellar crystal could be also confirmed by using the changes of intensity of (002). Furthermore, the streaking reflections of (101) and (111) in Fig. 4(a) and (f) changed to the four-point pattern as shown in Fig. 4(c) and (d). This result also suggests the lamellar rotation in the same case of (020) reflection.

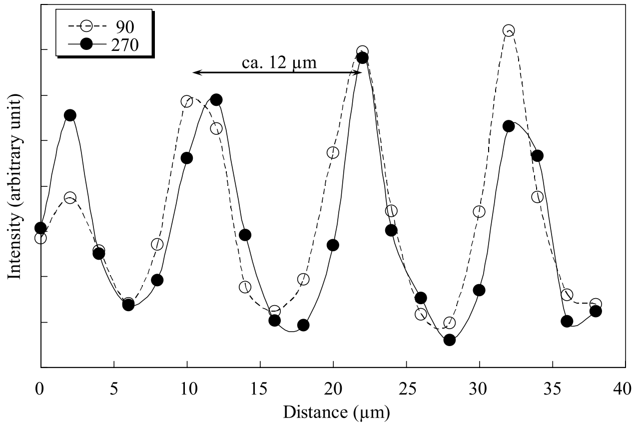

Fig. 6. Normalized integrated intensity of (020) reflections as a function of the scanned distance for P(3HB-*co*-8%-4HB) spherulite.

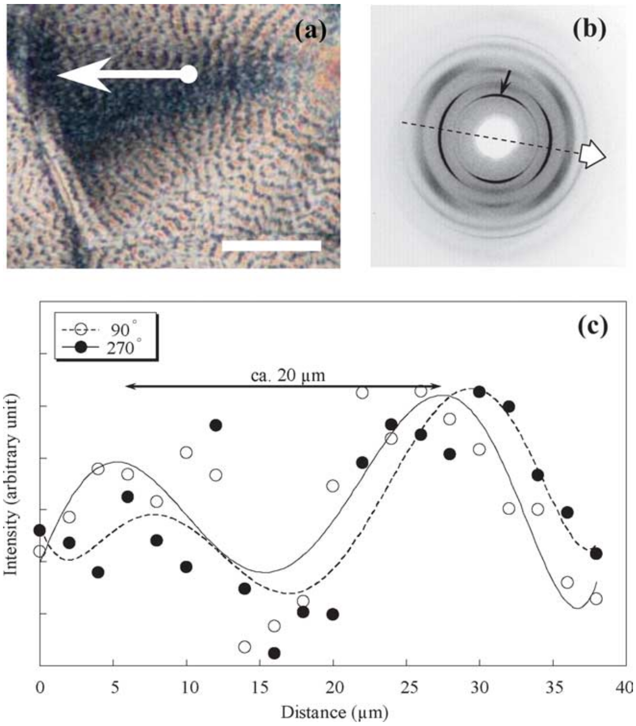

Fig. 7. (a) Polarized optical micrograph of P(3HB-*co*-8%-3HH) spherulite investigated by microbeam X-ray diffraction. The arrow shows the position where the scan was performed. The scale bar is 200 μm. (b) Microbeam X-ray diffraction pattern of P(3HB-*co*-8%-3HH) spherulite along the spherulite radius. The black and white arrows indicate the (020) reflection and the growth direction of spherulite defined as 0° for azimuthal angle, respectively. (c) Normalized integrated intensity of (020) reflections as a function of the scanned distance for P(3HB-*co*-8%-3HH) spherulite.

In the cases of P(3HB-*co*-8%-3HH) and P(3HB-*co*-10%-6HH) spherulites (110 μm and 90 μm thickness), the optical micrographs and X-ray diffraction patterns during scanning along the radial direction are shown in Figs. 7 and 8, respectively. The band spacings of P(3HB-*co*-8%-3HH) and P(3HB-*co*-10%-6HH) spherulites were 20 and 25 μm, respectively. A 40 μm distance was scanned in 2 μm steps as shown in Figs. 7(a) and 8(a). X-ray diffraction diagrams of P(3HB-*co*-8%-3HH) and P(3HB-*co*-10%-6HH) spherulites were same as those of P(3HB-*co*-8%-4HB) spherulite. The intensities of azimuthal angles of (020) reflection for P(3HB-*co*-8%-3HH) and P(3HB-*co*-10%-6HH), marked by arrows in Figs. 7(b) and 8(b), changed periodically as a function of the distance as shown in Figs. 7(c) and 8(c), respectively. The both oscillation periods obtained from X-ray diffraction diagrams for P(3HB-*co*-8%-3HH) and P(3HB-*co*-10%-6HH) spherulites were ca. 20 μm. This value corresponds to band spacings observed by optical

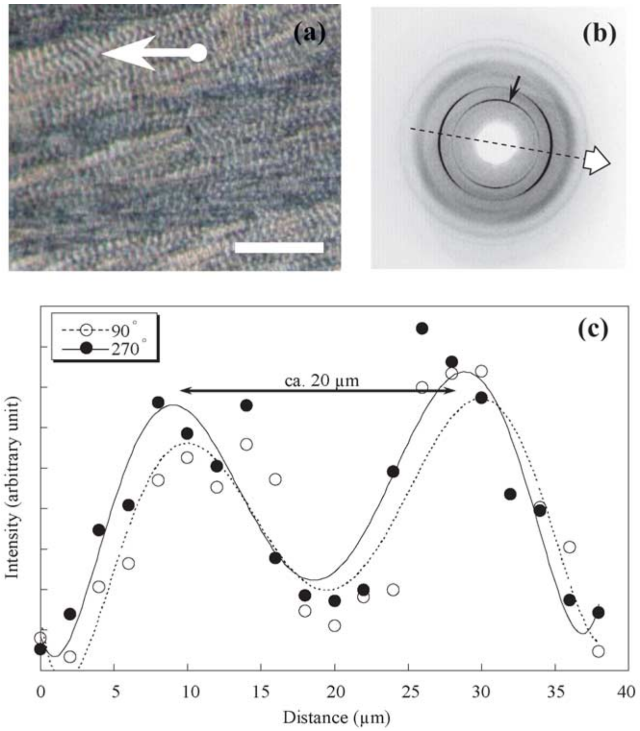

Fig. 8. (a) Polarized optical micrograph of P(3HB-*co*-10%-6HH) spherulite investigated by microbeam X-ray diffraction. The arrow shows the position where the scan was performed. The scale bar is 200 μm. (b) Microbeam X-ray diffraction patterns of P(3HB-*co*-10%-6HH) spherulite along the spherulite radius. The black and white arrows indicate the (020) reflection and the growth direction of spherulite defined as 0° for azimuthal angle, respectively. (c) Normalized integrated intensity of (020) reflections as a function of the scanned distance for P(3HB-*co*-10%-6HH) spherulite.

microscopy of ca. 20 μm for P(3HB-*co*-8%-3HH) and ca. 25 μm for P(3HB-*co*-10%-6HH)), respectively. Therefore, these results also indicate that the oscillation period obtained from the change of intensities shows periodically structural variation, even if the band spacing is very narrow and sample thickness is thick.

Our results in this experiment do not allow to directly discuss about the rotation of one lamellar crystal as like the edge-on and flat-on crystal features in twisting lamellae, because the lamellar thickness and long period in P(3HB) copolymers spherulites are known about 2–3 and 7–9 nm, respectively [11]. The periodic structural change obtained from intensity in this study shows a total structural change reflected from lamellar stacks in spherulite. Based on the results that the lamellar crystals rotated along the radial direction in P(3HB) spherulite and all oscillation period in spherulites of P(3HB) copolymers match regularly with band spacing, the lamellar crystals in spherulites of P(3HB) copolymers are also considered to twist as like P(3HB) spherulite. Therefore, these results obtained by microbeam X-ray diffraction seem to detect clearly the torsional changes of lamellae in spherulites with narrow band spacing of 15–25 μm.

4. Conclusions

Lamellar twisting in spherulites of P(3HB) and its copolymers with narrow band spacing of 15–25 μm was investigated by a microbeam X-ray diffraction in synchrotron radiation. Radial scanning of spherulites in 2–4 μm steps with 0.5 μm diameter of fine microbeam were performed. The *a*-axis lay along the radial direction, while the *b*- and *c*-axes rotated around *a*-axis in spherulites. The periodicity of reflection intensity obtained by microbeam X-ray diffraction was in good agreement with the narrow band spacing measured by polarized optical microscopy for P(3HB) copolymers spherulites. These results indicate a twisting of lamellar crystal in spherulites with narrow band spacing. The microbeam X-ray diffraction in synchrotron radiation could be demonstrated as a very useful and novel method to monitor the torsional changes of lamellar crystals and to analyze concentric alternating extinction banded spherulites.

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