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Electron and X-ray diffraction study on poly(4-hydroxybutyrate)

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Abstract

Lozenge-shaped poly(4-hydroxybutyrate) single crystals, showing spiral growth, were obtained from a dilute ethanol solution. The crystals gave well-resolved electron diffraction patterns from which the reciprocal lattice parameters $a^* = 1.305\ \mathrm{nm}^{-1}$, $b^* = 2.085\ \mathrm{nm}^{-1}$ and $\gamma^* = 90°$ could be determined. Systematic absences occurred at every odd reflection along the two orthogonal axes a^* and b^*. Therefore, the P(4HB) electron diffraction pattern is consistent with a $p2gg$ two-dimensional symmetry. The unit cell of P(4HB) is orthorhombic with space group $P2_12_12_1$ and lattice constants $a = 0.775 \pm 0.002$ nm, $b = 0.477 \pm 0.002$ nm and c (fiber axis) $= 1.199 \pm 0.002$ nm, which is determined by X-ray fiber diagram of a stretched-annealed film combined with electron diffraction patterns of single crystals. There are two chains in one unit cell, which exist in antiparallel arrangement.

Keywords: Poly(4-hydroxybutyrate); Single crystals; Electron and X-ray diffraction

The family of microbial polyesters known as polyhydroxyalkanoates (PHAs) has been receiving considerable attention due to their potential use as an environmentally friendly thermoplastic [1]. Most of the work has been done to elucidate the structure and degradation mechanism of poly([*R*]-3-hydroxybutyrate) (P(3HB)) and its copolymers [2–11]. The results obtained are crucial for the design and synthesis of PHAs that can biodegrade in a predetermined time and manner. Among the various PHAs, poly(4-hydroxybutyrate) (P(4HB)) and its copolymers have been identified as one of the promising candidate with attractive properties [12,13]. In addition, P(4HB) is currently also receiving much attention in the medical field because of its superior properties as well as biocompatibility [14,15].

In contrast to P(3HB), however, the crystal structure of P(4HB) has not been studied in detail, mainly because of difficulties in producing highly pure samples. Recently, substantial amounts of P(4HB) homopolymers have been produced by genetically engineered bacteria [16,17]. In the preliminary studies carried out by Mitomo et al. [18] and Pazur et al. [19], they reported the X-ray fiber diagram of P(4HB) from the necking-stretched and annealed film. The unit cell of P(4HB) is orthorhombic with space group $P2_12_12_1$ and parameters: $a = 0.775$ nm, $b = 0.479$ nm and c (fiber axis) $= 1.194$ nm. However, a detailed analysis of X-ray fiber diagram concerning lattice spacing data and three-dimensional structure has not yet been performed. Neither have the preparation and investigation of single crystals of P(4HB) been reported.

In this communication, lozenge-shaped single crystals of P(4HB) were obtained from a dilute solution by isothermal crystallization, and the morphology and structure of the single crystals were studied by means of transmission electron microscopy (TEM).

The P(4HB) sample used in this study (weight-average molecular weight (M_w) = 96 000 and polydispersity (DPI) = 2.1) was obtained through alkaline-hydrolysis of a bacterial P(4HB) (M_w = 963 000 and DPI = 2.6), which was produced using a recombinant *Comamonas acidvorans* (JCM 10181) [17].

The hydrolyzed P(4HB) was dissolved in ethanol at 140°C for 30 min in a pressure-resistant glass tube and then crystallized at 80°C for 16 h to grow the single crystals. The crystal suspension was deposited onto carbon-coated copper grids for electron diffraction study. For image observation, the samples were shadowed with Pt–Pd alloy to enhance the contrast. A JEM-2000FX II electron microscope was operated at an acceleration voltage of 120 kV for electron diffraction and image study. Images and electron diffraction diagrams were recorded on Mitsubishi MEM films.

Fig. 1a shows the transmission electron micrograph of P(4HB) single crystals grown in ethanol at 80°C for 16 h. They appear as lozenge-shaped crystals with screw dislocation and the ratio between the two diagonal axes of the lozenge-shaped crystals was about 3:5 (minimum:maximum). Electron

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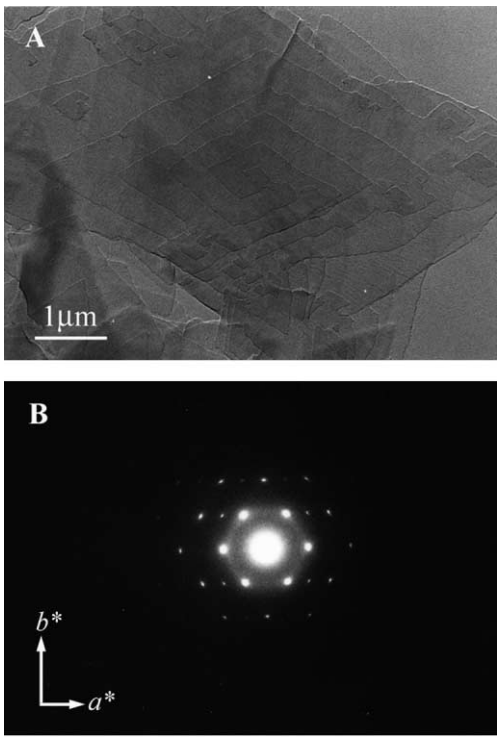

Fig. 1. (A) Electron micrograph of lozenge-shaped lamellar single crystals of P(4HB) with screw dislocations and (B) typical (*hk*0) electron diffraction diagram.

diffraction patterns were recorded from selected areas, yielding diffractograms as shown in Fig. 1b. The diagram contains 14 independent reflections that are mirrored in the four quadrants defined along the two orthogonal reciprocal axes a^* and b^*. Along these axes, systematic absences occurred at every odd reflection, which suggests that the diagram correspond to a *p*2*gg* symmetry. The reciprocal lattice parameters, a^* and b^*, were refined with a least-squares procedure applied to the observed *d*-spacing of all diffraction spots, and the calculation gave $a^* = 1.305\ \text{nm}^{-1}$, $b^* = 2.085\ \text{nm}^{-1}$ and $\gamma^* = 90°$. The observed *d*-spacings and intensities of the electron diffraction diagram of the P(4HB) single crystal are summarized in Table 1. Based on the triple-exposure of the selected-area electron diffraction, and the normal and selected-area images, it was confirmed that the growth planes of the single crystals corresponded to the crystallographic {110} planes. By using atomic force microscopy, the crystal thickness was determined to be 7–8 nm. Considering the fiber repeat distance [18,19] and the molecular weights, the chain foldings occur at the surfaces of P(4HB) single crystals as in polyethylene [20,21] and poly([*R*]-3-hydroxyvalerate) [22] single crystals, and the folding planes are parallel to {110} planes.

X-ray diffraction was performed on P(4HB) cast film that has been stretched to 550% prior to annealing at 55°C for seven days to increase the crystallinity. A Rigaku-UltraX18-Rint X-ray generator operating at 40 kV and 200 mA was used to obtain the X-ray diffraction. An X-ray beam of Cu–Kα of wavelength 0.1542 nm was used under vacuum condition. Calcium fluoride ($d_{111} = 0.3154$ nm) was used for calibration.

X-ray fiber diagram shows five layer lines and about 25 reflections in one quadrant as in Fig. 2. All the equatorial reflections of the X-ray fiber diagram are observed in electron diffraction pattern, which confirms that the electron diffraction diagram is a projection along the *c*-axis; that is, the polymer chains align perpendicular to the lamellar base of the crystal. The X-ray pattern has a lower resolution than the electron diffraction pattern, which displays diffraction spots down to 0.128 nm spacing as opposed to only 0.203 nm for the X-ray pattern. The existence of even order reflections along the meridian suggests the presence of a two-fold screw axis along the crystallographic *c*-axis direction. Combined with the *p*2*gg* symmetry from the electron diffraction pattern of single crystal, it can be concluded that P(4HB) crystals have the orthorhombic $P2_12_12_1$ space group. Based on careful measurements of X-ray and electron diffraction patterns, the lattice constants are determined to be: $a = 0.775 \pm 0.002$ nm, $b = 0.477 \pm 0.002$ nm and $c = 1.199 \pm 0.002$ nm with a least-squares procedure. The observed and calculated *d*-spacings and intensities for the X-ray fiber diagram are summarized in Table 1. The unit parameters determined here are consistent with those reported by Mitomo et al. [18] ($a = 0.775$ nm, $b = 0.479$ nm and c (fiber axis) $= 1.194$ nm) within experimental error.

The calculated density for this unit cell is $1.29\ \text{g cm}^{-3}$, which is fairly close to the observed density, $1.22\ \text{g cm}^{-3}$, determined by flotation in a NaBr aqueous solution. Density considerations lead to two chains per unit cell, packed in an antiparallel arrangement where each chain contains two residues.

Mitomo et al. calculated the fiber repeat distance of an all-trans conformation to be 1.204 nm based on all bond angles of 109.5° and bond lengths of C–C(O) = 0.151 nm, C–C = 0.154 nm, C–O = 0.143 nm and C(O)–O = 0.137 nm [18]. Thereafter, Kobayashi et al. reported the

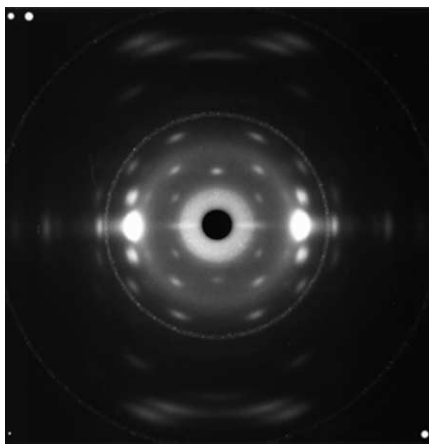

Fig. 2. X-ray fiber diagram of P(4HB) film stretched to 550% at room temperature and annealed at 55°C for seven days. The Debye–Scherrer ring of CaF_2 was recorded for calibration purposes.

Table 1
Observed and calculated *d*-spacings from X-ray fiber diagram of P(4HB) stretched-annealed film and electron diffraction pattern of single crystal

Index[a] *hkl*	*d*-spacing (nm)			Intensity[b]	
	Calculated	X-ray (observed)	Electron (observed)	X-ray	Electron
100	0.775	–[c]	–		
010	0.478	–	–		
110	0.406	0.410	0.408	vs	vs
200	0.387	0.382	0.385	vs	vs
210	0.301	0.300	0.299	s	m
300	0.258	–	–		
020	0.238	0.239	0.238	w	m
120	0.228		0.229	m	m
310	0.227	0.227	0.225	w	m
220	0.203	0.203	0.204		m
400	0.194		0.192		m
410	0.179		0.178		vw
320	0.175		0.175		w
420	0.150		0.150		w
510	0.147		0.145		w
330	0.135		0.137		vw
600	0.125		0.128		vw
001	1.199	–			
101	0.651	0.648		m	
011	0.443	0.444		w	
111	0.385	0.386		s	
201	0.369	0.367		s	
211	0.292	0.291		w	
002	0.599	0.600		s	
102	0.474	0.477		m	
012	0.373	0.372		vw	
112	0.336	0.334		m	
212	0.269	0.269		vw	
003	0.400	–			
103	0.355	0.353		m	
113	0.285	0.284		vw	
104	0.279	0.280		vw	
204	0.237	0.240		w	
214	0.212	0.212		vw	
105	0.229	0.228		w	
015	0.214	0.214		m	
205	0.204	0.204		m	
215	0.187	0.187		vw	

[a] Indexed in terms of an orthorhombic unit cell with parameters $a = 0.775$ nm, $b = 0.477$ nm, and c (fiber axis) $= 1.199$ nm.
[b] vs = very strong; s = strong; m = medium; w = weak; vw = very weak.
[c] Systematic absence.

slightly deviated planar ziazag conformation and preliminary packing-state of molecular chains [23]. On the other hand, Pazur et al. reported the fiber repeat of an all-trans chain of P(4HB) was 1.240 nm [19]. They performed energy calculation on a single chain of P(4HB) and found five minimum-energy conformers. Among them, one conformer possessing a pitch of 1.19 nm is in good agreement with the observed fiber diagram pitch. The observed fiber repeat distance of 1.199 nm in this work does not involve the fully extended chain with a repeat of 1.204 nm [18] or 1.240 nm [19], and therefore, the molecule is slightly twisted in the unit cell.

For aliphatic polyesters of the type $[-(CH_2)_m-CO-O-]_n$, when the number of methylene is even, such as poly-β-propiolactone ($m = 2$) [24] and poly-δ-valerolactone ($m = 4$) [25], the molecular conformation in single crystals is all-trans. On the other hand, when the number of methylene is odd, such as P(4HB) ($m = 3$) and poly(ϵ-caprolactone) ($m = 5$) [26–28], the molecular conformation in single crystals is close to an all-trans conformation, in fact, a 2_1 helix because there is a slight reduction in the all-trans c repeat value.

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