Composition Range of Crystal Phase Transition of Isodimorphism in Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

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Introduction

Poly(3-hydroxybutyrate) (P(3HB)) and its copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) can be isolated with a very high purity from the cells of some bacteria by solvent extraction.[1,2] It has been reported that a structural characteristic of P(3HB-*co*-3HV) is isodimorphism, i.e., P(3HB-*co*-3HV) crystallizing either in a P(3HB) or P(3HV) crystal lattice for the HV contents lower or higher than ca. 40 mol %, respectively.[3,4] Recently, it has been reported that the coexistence of both crystal phases and pseudoeutectic behavior occur at a narrow composition range, i.e., at a composition of around 32 mol % for synthetic P(3HB-*co*-3HV)[5] or 41 mol % for bacterial P(3HB-*co*-3HV).[6,7] The cocrystallization of these copolymers has been interpreted theoretically with thermodynamic treatment.[8-10]

In the present study, the composition range of crystal phase transition, where the pseudoeutectic or both P(3HB) and P(3HV) crystal phases coexist, was investigated for the samples fractionated with a solution of an acetone–water system by wide-angle X-ray scattering and differential scanning calorimetry.

Experimental Section

Copolymers P(3HB-*co*-3HV) containing 34.2, 45.7, 64.2, and 67.8 mol % HV were used, which were isolated from *Alcaligenes eutrophus* (ATCC 17699). The microorganism was first grown at 30 °C in nutrient-rich medium (100 mL) containing 1 g of yeast extract, 1 g of polypeptone, 0.5 g of meat extract, and 0.5 g of $(NH_4)_2SO_4$. The cells were harvested after 24 h and washed with water. At this stage, accumulation of polyesters in the cells was not observed. To promote polyester synthesis, about 0.4-g quantities of the washed cells were transferred into a nitrogen-free medium[11] containing 2 g of different ratios of two carbon sources, i.e., butyric and valeric acids. The cells were cultivated in these media (100 mL, pH = 7.0) for 48 h at 30 °C, harvested by centrifugation, washed with acetone, and finally dried under vacuum at room temperature. Polyesters were extracted from the dried cells with hot chloroform in a Soxhlet apparatus and purified by reprecipitation with hexane.[2]

The procedure of fractionation of these samples was as follows: the samples were dissolved with hot acetone and then kept at 8 °C for 20 h followed by filtration or centrifugation to isolate the precipitate. Next, the solution was diluted with water to a concentration of 95% acetone and kept at the same condition followed by isolation. The same procedure was repeated to the concentration, lowering by every 5% step.

The melting behavior of a 3-mg sample was studied by using a Perkin-Elmer Model DSC-7 differential scanning calorimeter (DSC) at a heating rate of 10 °C/min under a nitrogen atmosphere. The melting peak temperature, after being corrected for the thermal lag (or heating rate dependence) and calibrated with high-purity standards, was defined as the melting point T_m with accuracy within ±0.1 °C.

The wide-angle X-ray scattering (WAXS) pattern was recorded photographically using a cylindrical vacuum camera (50 mm ϕ) by the method of rotating the needle-shaped sample. Nickel-filtered Cu Kα X-ray beams with a pinhole collimator from a Rigaku-Denki Rotaunit RU-200 (40 kV, 150 mA) were used. The diffraction pattern was obtained from the microdensitometer trace along the equatorial direction.

The ^1H and ^{13}C NMR analyses of the fractionated samples were carried out on a JEOL GSX-270 spectrometer in the pulsed Fourier transform (FT) mode. The measurement conditions were almost similar to those of the other report.[12]

The GPC chromatograms were recorded with an HLC-802A high-performance liquid chromatograph (Tosoh Co., Ltd.) at 38 °C equipped with a series of four columns of TSK gel and an RI-8 differential refractometer. The eluent was chloroform with a flow rate of 1 mL/min, and the polymer concentration was ca. 1% (w/v). The number-average molecular weight $\bar{M}_n$ was calibrated using polystyrene standards and six different $\bar{M}_n$ values of PHB samples (from 4.29×10^3 to 5.64×10^5) evaluated by GPC and a low-angle laser light scattering (GPC-LALLS) system.

Results and Discussion

Figure 1 shows typical WAXS photographs of P(3HB) and the copolymers P(3HB-*co*-45.7 mol % 3HV) and P(3HB-*co*-64.2 mol % 3HV) (hereinafter these copolymers are called samples I and II, respectively) and P(3HB-*co*-67.8 mol% 3HV) using a cylindrical camera. The latter copolymer shows only the P(3HV) phase, while samples I and II show the mixed pattern of P(3HB) and P(3HV) crystal phases. If both samples are mixtures of several copolymers having HV contents lower or higher than ca. 40 mol %,[13] it is natural that they show the mixed WAXS patterns of both P(3HB) and P(3HV) crystal phases. Therefore, we carried out the fractional precipitation methods for both samples I and II using a solution of an acetone–water system. The results of fractionation of both samples and characterization of the fractionated samples were listed in Table I. The composition of the HV component (mol %) was estimated from ^1H NMR study. In Table I, we can see that both samples I and II are the mixtures of several copolymers having different compositions. In order to determine the randomness in sequence distribution, it is convenient to use a new parameter D defined as $D = F_{VV}F_{BB}/(F_{VB}F_{BV})$ (where the subscripts of B and V present the HB and HV units, respectively, and F_{XY} presents the mole fraction of the XY sequence estimated from the ^{13}C NMR spectrum), which has been proposed by Kamiya et al.[12] If a copolymer is statistically random, D is close to 1 and larger or smaller than 1 for a block or an alternate copolymer, respectively. All samples fractionated with the concentration of aqueous acetone less than 95% are typical random copolymers because D values are close to 1.

Figure 2 shows DSC heating curves for the fractionated samples. Sample I-1 shows a main melting peak at 174 °C, which corresponds to P(3HB) homopolymer, accompanied by several small peaks at lower temperatures. This implies that sample I-1 is a mixture of P(3HB) (major content) and a minor content of several copolymers having different HV composition, which is the reason why sample I-1 showed the very large D value and very low HV

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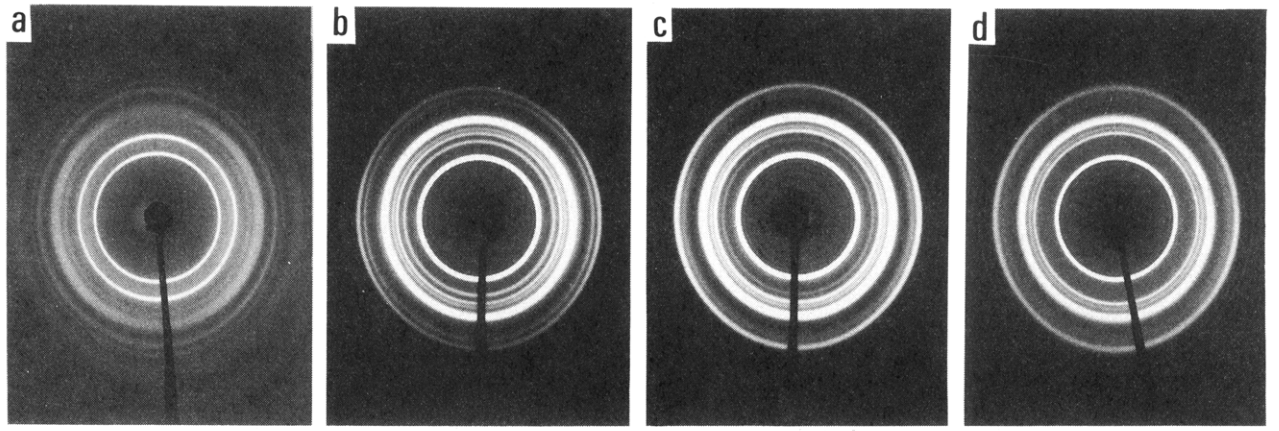

Figure 1. Typical WAXS photographs of P(3HB) (a), P(3HB-*co*-45.7 mol% 3HV) (b), P(3HB-*co*-64.2 mol % 3HV) (c), and P(3HB-*co*-67.8 mol % 3HV) (d) using a cylindrical camera. Photographs of a and d show typical P(3HB) and P(3HV) crystal phases, respectively, while those of b and c show the mixed diffraction patterns of P(3HB) and P(3HV) crystals.

Table I. Fractionation and Characterization of Samples I and II

sample	conc of aq acetone (%)	fraction (wt %)	HV mol %	T_m (°C)	$\bar{M}_n \times 10^{-5}$	$\bar{M}_w/\bar{M}_n$	D
I			45.7	72	2.65	3.44	1.48
I-1	100	27	23.0	84, 132, 174	3.14	3.60	29.6
I-2	90	19	71.6	82	2.63	3.06	1.15
I-3	85	42	44.6	70	2.48	3.31	1.03
I-4	80	4	40.9	69	1.96	2.93	0.82
II			64.2	94	2.82	2.90	1.61
II-1	100	12	85.0	95	2.93	2.83	2.26
II-2	95	5	78.1	93	2.91	2.88	1.30
II-3	90	14	72.2	85	2.94	2.82	0.93
II-4	85	36	55.2	79	2.80	2.44	1.06
II-5	80	11	48.2	73	2.39	2.42	1.09
II-6	70	16	44.5	71	1.81	2.55	0.91

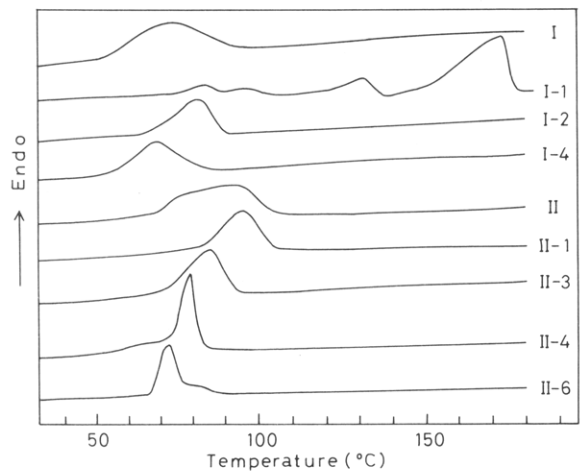

Figure 2. DSC heating curves of the fractionated samples I and II.

composition. Therefore, sample I-1 should be excluded from the following discussion. All the other samples seem to be the copolymers of single composition, because they show single and relatively sharp melting peaks. Samples I-2, I-3, and I-4 show peaks at 82, 70, 69 °C, respectively. The fractionated samples of the II series show single and sharp melting peaks at a temperature range from 95 to 71 °C. It was observed that T_m decreases to the minimum value around 69 °C and the content of HV mol % converges to a composition of ca. 40% as the concentration of acetone decreases. The number-average molecular weight $\bar{M}_n$ of the fractionated sample slightly decreased with decreasing the concentration of acetone, while $\bar{M}_n$ of the sample fractionated at extremely low concentration of acetone, i.e., 80 or 70%, decreased considerably, reflecting the increment in solubility of the sample.

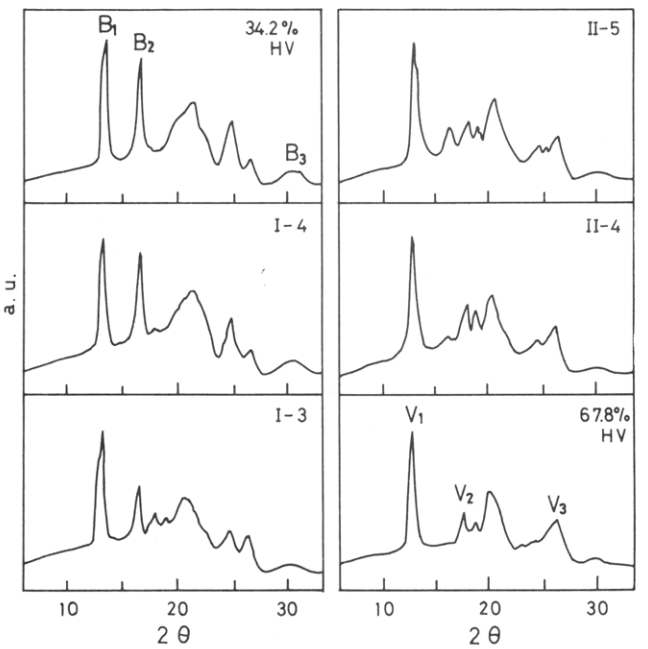

Figure 3. Typical WAXS patterns of P(3HB-*co*-34.2 mol % 3HV), P(3HB-*co*-67.8 mol % 3HV) and the fractionated samples I-4, I-3, II-5, and II-4. The diffraction peaks B_1, B_2, and B_3 were assigned to the (020), (110), and (002) planes of the P(3HB) lattice, and the peaks V_1, V_2, and V_3, to the (110), (020), and (211) planes of the P(3HV) lattice, respectively. All four fractionated samples show the mixed patterns of P(3HB) and P(3HV) crystal phases.

Typical WAXS patterns of these samples are shown in Figure 3, where the samples of HV contents of 34.2 and 67.8 mol % show P(3HB) and P(3HV) crystal phases, respectively. Sample I-1 showed the P(3HB) crystal phase, while samples I-2, II-1, II-2, and II-3 showed the P(3HV)

crystal phase. All other samples I-3, I-4, II-4, and II-5 show the mixed pattern of both P(3HB) and P(3HV) crystal phases as shown in Figure 3 (sample II-6 also showed the mixed pattern). The characteristic diffraction peaks of the P(3HV) crystal phase (denoted as peaks V_1, V_2, and V_3) increased in their intensities with increasing the HV content. In Figure 3, both samples I-4 and II-4 whose HV contents are lowest and highest show the smallest diffraction peaks of P(3HV) and P(3HB) crystal phases, respectively.

Consequently, samples I and II were demonstrated to be mixtures of several random copolymers having different compositions. The fractionated samples whose HV content range was 40.9–55.2 mol % showed the coexistence of both P(3HB) and P(3HV) crystal phases. The crystal phase transition of isodimorphism[3] for P(3HB-*co*-3HV), therefore, occurred rather at the broad composition range from 40.9 to 55.2 mol % HV. We could ascertain this fact with some other fractionated copolymers of HV contents lying between this range, which always showed the same mixed patterns of P(3HB) and P(3HV) crystal phases (data not shown). At this region, the lattice indices of the P(3HB) phase expanded up to the maximum values for P(3HB-*co*-55.2 mol % 3HV) (a = 0.602 nm; b = 1.343 nm; c = 0.604 nm) from the original indices of P(3HB) (a = 0.576 nm; b = 1.320 nm; c = 0.596 nm),[14,15] whereas those of the P(3HV) phase remained almost unchanged because the side group of the HB unit is smaller than that of the HV unit,[13] though Scandola et al. reported the P(3HV) crystal lattice slightly contracts with an increasing amount of HV units.[6]

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