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Crystalline Structural Change of Bacterial Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) with Narrow Compositional Distribution

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Bacterial poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] is a statistically random and highly crystalline biodegradable copolyester.[1,2] In general, comonomer composition as well as chemical structure of crystalline copolymer affect degree of crystallinity and properties, such as thermal behavior, mechanical strength, biodegrability and so on. In almost all crystalline copolymers, the minor comonomer unit would interrupt the crystallization behavior of the major comonomer component and the former is excluded from the crystalline region, resulting in the decrease of degree of crystallinity.[3] In the case of bacterial P(3HB-*co*-3HV), on the other hand, it is well-known that the minor comonomer unit is included as the crystal constituent in the crystal lattice of the major comonomer unit.[3−6] This phenomenon is called as cocrystallization. Because of cocrystallization, P(3HV-*co*-3HV) maintains high degree of crystallinity of more than ca. 50%, over the whole range of comonomer composition. It has been reported that 3HB-rich and 3HV-rich P(3HB-*co*-3HV)s form P(3HB) and P(3HV) homopolymer-type crystalline lattice, respectively.[2−7] The coexistence of both P(3HB) and P(3HV) homopolymer-type crystalline lattice has been observed by X-ray diffraction for P(3HB-*co*-3HV) samples in a relatively broad comonomer composition range between ca. 36−56 mol % 3HV.[8] But up to now, it has not been clarified whether bacterial P(3HB-*co*-3HV) samples show a crystalline structural change at the distinct comonomer composition or not. The difficulty in the determination of the strict comonomer composition range for the crystalline structural change is due to the fact that bacterial copolyester samples have broad and complex compostional distributions.[8−11] In fact, the morphology and some physical properties of bacterial copolyesters should be characterized as the blends of copolyesters with different comonomer composition.[10]

In this note, we investigate the crystalline lattice structures of bacterial P(3HB-*co*-3HV)s by differential scanning calorimetry (DSC) and wide-angle X-ray diffractometry (WAXD) by using the compositionally well fractionated P(3HB-*co*-HV) samples.

The P(3HB-*co*-3HV) samples were biosynthesized by fermentation of *Ralstonia eutropha* from butyric acid and valeric acid as carbon sources[1,2] and were extracted using chloroform solvent. The crude P(3HB-*co*-3HV) samples were purified by precipitation using chloroform/methanol, and then using chloroform/*n*-hexane. Bacterial as produced original sample was fractionated into fractions with different 3HV contents via fractional precipitation method by adding stepwise *n*-heptane to the chloroform solution of copolymer[9] (starting polymer concentration 1 g/100 mL). The P(3HB-*co*-3HV) films cast from chlorofolm solution were melt at 195 °C and isothermally crystallized at 30 °C for at least 4 weeks before the measurements of DSC and WAXD.

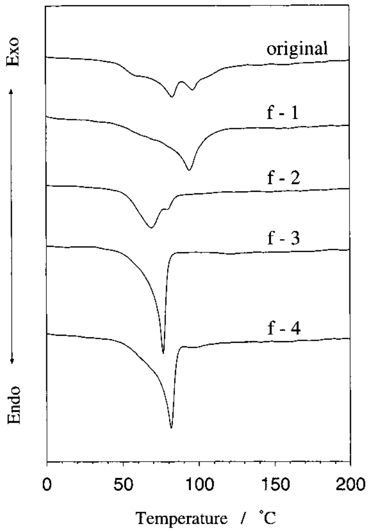

Figure 1. DSC thermograms measured under heating rate of 10 °C/min for isothermally crystallized unfractionated original P(3HB-*co*-3HV) sample with 45 mol % 3HV and compositionally fractionated samples.

^{1}H NMR spectra in $CDCl_3$ solution were recorded on a JEOL GSX-270 spectorometer at 270 MHz and 30 °C.

Weight-average molecular weight M_w and polydispersity M_w/M_n were characterized by a Tosoh HLC-8020 GPC system with a Tosoh SC-8010 controller, refractive detector, TSK GEL G2000Hxl and GMMHxl columns. Polystyrene samples with narrow molecular-weight distribution were used as standards to calibrate the GPC curve.

DSC measurements were performed on a Seiko DSC-20 assembled with a SSC-580 thermal controller. Isothermally crystallized polymer samples were pre-sealed in aluminum pans. DSC thermograms were recorded from −30 to +210 °C at heating rate of 10 °C/min.

WAXD patterns were recorded in a θ range of 5−50° at scanning speed of 1°/min on a Rigaku RU-200 (50 kV/180 mA). The nickel-filtered Cu Kα X-ray radiation ($\lambda = 0.154$ 18 nm) were used as the source. The degrees of crystallinity were estimated according to the method of Vonk.[12]

During chloroform/*n*-heptane fractionation procedures as described in the Experimental Section, the 3HV mol % content of precipitated fraction increased with an increase of *n*-heptane composition in mixed solvent.[9]

One of the bacterially as-produced original P(3HB-*co*-3HV) samples with 45 mol % 3HV was fractionated into 10 fractions with 26−60 mol % 3HV (*n*-heptane composition in the mixed solvent ranged from 60 to 78 vol %) and that with 47 mol % 3HV fractionated into 8 fractions with 26−56 mol % 3HV (*n*-heptane composition from 62 to 78 vol %). The fractionation was confirmed to occur mainly due to difference in comonomer content, but the effect of molecular weight difference could be negligible except the last one or two fractions.[9] In this report, one original P(3HB-*co*-3HV) with 45 mol % 3HV and four fractions, designated f-1,

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Table 1. Properties of Original and Compositionally Fractionated Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)s Bacterially Synthesized by *R. eutropha* from a Butyric Acid/Valeric Acid Mixture

sample	3HV content[c] in mol %	M_w[d] × 10^5	M_w/M_n[d]	T_m/°C[e]	lattice type[f]	crystallinity[f] in%
original	47	10.0	2.85	ca. 59, 75	HB, HV	50
original	45	5.17	2.66	59.5, 83.0, 97.2	HB, HV	50
f-1[a]	33	4.64	1.62	94.3	HB	48
f-2[a]	47	11.7	2.04	68.7, 79.2	HB	50
f-3[b]	52	12.2	1.65	76.4	HV	50
f-4[a]	56	5.26	2.40	81.4	HV	52

[a] Fraction obtained from original P(3HB-*co*-3HV) with 45 mol % 3HV. [b] Fraction obtained from original P(3HB-*co*-3HV) with 47 mol % 3HV. [c] Contents of 3HV mol % were determied by the relative intensities of the methyl resonances of 3HB and 3HV units in ^{1}H NMR spectra. [d] Weight-average molecular weight (M_w) and polydispercity (M_w/M_n) were determined from GPC using polystyrene standards. [e] Melting temperature (T_m) was taken as the top of DSC melting peak. [f] Crystalline lattice type and the degree of crystallinity were determined by WAXD.

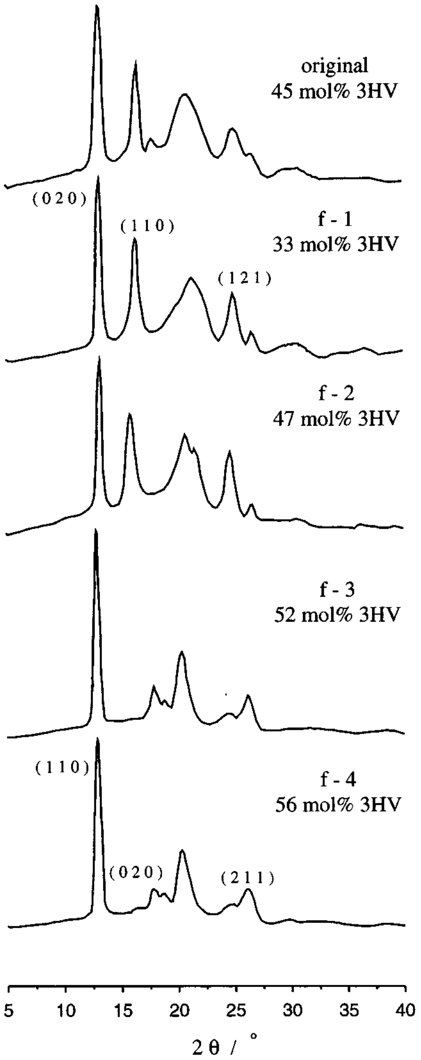

Figure 2. Wide-angle X-ray diffraction patterns of unfractionated original sample and compositionally fractionated samples.

f-2, f-3, and f-4, obtained from two original P(3HB-*co*-3HV)s with 45 mol % and 47 mol % 3HV were used as samples. Properties of samples are summarized in Table 1.

Although the average 3HV content of unfractionated original and those of fractions f-2 and f-3 are quite similar to each other in a range of 45−52 mol %, DSC thermal behavior of these samples is quite different, as shown in Figure 1. In this figure, broad and multiple peaks are observed for the unfractionated original samples, while fractions f-3 and f-4 exhibit sharp single melting peaks. Sample f-2 also shows a distinct melting peak with a small shoulder. The fractions obtained by solvent/nonsolvent fractionation are considered to have very narrow compositional distribution, because they show very sharp DSC melting peaks.

WAXD patterns of these copolyesters are shown in Figure 2. The diffraction peaks corresponding to the P(3HB) and P(3HV) homopolymer-type crystalline lattices were observed at the same time in the unfractionated original P(3HB-*co*-3HV) with 45 mol % 3HV content as reported in the previous paper,[4,8] that is, the peak at $2\theta = 17°$ corresponding to the (110) diffraction of the P(3HB) type lattice[13] and that at $2\theta = 18°$ to the (020) of the P(3HV) type lattice.[14] The fractions f-1 and f-2 show only the typical P(3HB) crystalline type WAXD patterns and the f-3 and f-4 show only the typical P(3HV) crystalline type WAXD pattern. Hence, the crystalline structures of f-2 and f-3 exist, being substantially isomorphous with those of P(3HB) and P(3HV) homopolymers, respectively, but both fractions having HB and HV monomer units in almost equal content. These results clearly indicate that the change of crystalline lattice type occurs in the very narrow range of 3HV composition between 47 and 52 mol % 3HV which is quite narrower than that found in the previous works.[8] The coexistence of both crystalline lattices observed previously for the P(3HB-*co*-3HV) samples with 3HV content ca. from 36 to 56 mol %[8] seems mainly due to the rather broad comonomer compositional distribution. The degree of crystallinity determined by WAXD were found to be almost constant ca. 50% for all fractionated as well as unfractionated original P(3HB-*co*-3HV) samples, indicating the occurrence of cocrystallization of 3HB and 3HV units in these samples.

Thus, it can be concluded that a little excess in the 3HB unit content of P(3HB-*co*-3HV) leads to the crystallization of 3HB and 3HV units into the P(3HB) homopolymer-type crystalline lattice, and that in the 3HV unit content leads to the crystallization of them into the P(3HV) homopolymer-type lattice.

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