Crystalline and Thermal Properties of Bacterial Copolyesters: Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)

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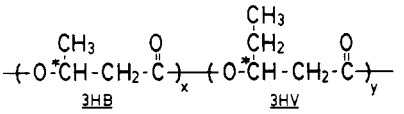
ABSTRACT: Two types of bacterial copolyesters, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), were analyzed by X-ray diffraction and differential scanning calorimetry. X-ray diffraction patterns of P(3HB-*co*-3HV) samples showed a high crystallinity (>50%) throughout a wide range of compositions from 0 to 95 mol % 3HV. The P(3HB-*co*-3HV) samples with compositions from 0 to 37 mol % 3HV crystallized in the P(3HB) lattice, while those with compositions from 53 to 95 mol % 3HV crystallized in the P(3HV) lattice. The X-ray crystallinities of P(3HB-*co*-4HB) samples decreased from 55 to 14% with increase of the 4HB fraction in the composition range of 0–49 mol % 4HB. The rates of crystallization of four polyester samples quenched from the melt decreased in the series P(3HB) ≃ P(3HB-*co*-83%3HV) > P(3HB-*co*-18%4HB) ≫ P(3HB-*co*-21%3HV). The thermal properties of 20 copolyester samples are reported.

Introduction

Optically active poly(3-hydroxybutyrate) (P(3HB)) accumulates in a variety of bacteria as an energy source.[1-5] The polyester can be isolated with a very high purity from the cells by solvent extraction.[2,6] The crystal structure of P(3HB) has been investigated by X-ray diffraction,[7,8] which indicated that P(3HB) molecule adopts a left-handed 2_1 helix with a fiber repeat of 0.596 nm. The thermal properties of P(3HB) have been reported by several authors.[9-11] One of the important characteristics of a bacterial polyester is in being thermoplastic with biodegradable properties.[12-14] An extracellular P(3HB) depolymerase has been isolated from *Alcaligenes faecalis*.[15]

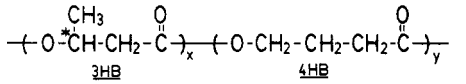
Imperical Chemical Ind. has developed a controlled fermentation for the production of an optically active copolyester of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) as



(1)

The copolyesters were isolated from *Alcaligenes eutrophus* grown in culture media containing propionic acid and glucose,[16] and the range of compositions was found to vary from 0 to 47 mol % 3HV.[17] The copolyesters have been shown to have a statistically random distribution of 3HB and 3HV units.[18,19] The random copolyesters exhibited approximately the same high degree of crystallinity (>60%) throughout a range of compositions up to 47 mol % 3HV, and only two crystal forms were detected by X-ray diffraction.[18] The copolyesters crystallized in either the P(3HB) unit cell or the P(3HV) unit cell, and the transformation from the P(3HB) lattice to the P(3HV) lattice occurred at about 30 mol % 3HV, which has led to the conclusion that the copolyester exhibits the unusual phenomenon of isodimorphism.[18]

Recently, we have found that the P(3HB-*co*-3HV) samples with a wide range of compositions from 0 to 95 mol % 3HV are produced in *A. eutrophus* by using pentanoic and butyric acids as the carbon sources.[20,21] In addition, some of us have recently succeeded in the fermentation production of a new copolyester of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) by *A. eutrophus* from 4-hydroxybutyric and butyric acids:[22,23]



(2)

The copolyesters were shown to have a statistically random distribution of 3HB and 4HB units, and the range of product compositions was found to vary from 0 to 49 mol % 4HB.[22]

In this paper we characterize two types of bacterial copolyesters, P(3HB-*co*-3HV) (3HV = 0–95 mol %) and P(3HB-*co*-4HB) (4HB = 0–49 mol %), by wide-angle X-ray diffraction and differential scanning calorimetry. The composition effects on the crystalline and thermal properties of bacterial copolyesters are reported.

Experimental Section

We isolated 12 samples of P(3HB-*co*-3HV) (3HV = 0–95 mol %) from *A. eutrophus* (NCIB 11599) grown in nitrogen-free mineral media containing pentanoic and butyric acids as the carbon sources.[20,21] The 3HV content in P(3HB-*co*-3HV) samples increased up to 95 mol % with increase of the fraction of pentanoic acid in the culture solution. The other 12 samples of P(3HB-*co*-4HB) (4HB = 0–49 mol %) were isolated from *A. eutrophus* (ATCC 17699) grown in nitrogen-free mineral media containing 4-hydroxybutyric and butyric acids.[22,23] The 4HB content in P(3HB-*co*-4HB) samples increased up to 49 mol % with increase of the fraction of 4-hydroxybutyric acid in the culture solution. The compositions of bacterial copolyesters were measured by ^{1}H NMR spectroscopy.[19,22]

Wide-angle X-ray diffraction measurements of copolyester samples were made on a Rigaku RAD-1VB system. Cu Kα radiation (λ = 0.1542 nm) was used as the source. The X-ray diffraction patterns of copolyester samples were recorded at 27 °C in the range 2θ = 6–40° at scan speed of 1–3°/min. X-ray crystallinities were measured for the polyester films that had been cast from chloroform solution and allowed to stand for 3 days at room temperature. Crystallization kinetics were measured for the melt-quenched samples that had been heated for 0.5 min at 185 °C and immediately quenched at room temperature. Crystallization was allowed to proceed at room temperature. Time zero was approximated as the time at which the sample was quenched. The percentage of crystallinity was calculated from diffracted intensity data according to Vonk's method.[24]

The melting temperatures of polyester samples were recorded on a Seiko DSC-10. The as-isolated powder samples of 3 mg were encapsulated in aluminum pans and heated at 10 °C/min up to 200 °C. The heat of fusion of indium (6.80 cal/g) was used as a calorimetric calibration. Where multiple endotherms were observed, the melting point from the higher temperature en-

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Table I
Degree of Crystallinity, Crystalline Structure, and *d* Spacings of P(3HB-*co*-3HV) Samples[a] from X-ray Diffraction

3HV, mol %	crystallinity, %	cryst struct	*d* spacings,[b] nm (020)	(110)	(002)	(211)
0	55 ± 5	P(3HB)	0.659	0.525	0.296	
9	58 ± 5	P(3HB)	0.664	0.532	0.294	
21	56 ± 5	P(3HB)	0.664	0.536	0.295	
37	52 ± 5	P(3HB)	0.663	0.546	0.298	
53	63 ± 5	P(3HV)	0.503	0.695		0.342
62	57 ± 5	P(3HV)	0.504	0.691		0.342
83	66 ± 5	P(3HV)	0.503	0.691		0.346
95	70 ± 5	P(3HV)	0.503	0.691		0.343

[a] Solution-cast films at 3 days after evaporation of the solvent ($CHCl_3$). [b] The deviations in these values are ±0.005 nm.

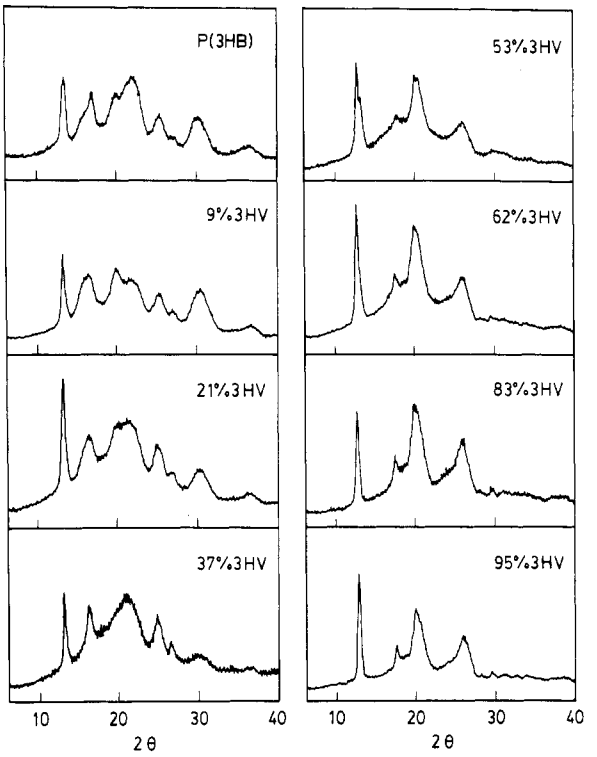

Figure 1. X-ray diffraction patterns of P(3HB) and P(3HB-*co*-3HV) films cast from $CHCl_3$ solution. Samples had been aged for 3 days at room temperature after evaporation of the solvent.

dotherm was taken as the true melting temperature.

Results and Discussion

P(3HB-*co*-3HV). Figure 1 shows the X-ray diffraction patterns of solution-cast P(3HB-*co*-3HV) films containing from 0 to 95 mol % 3HV. All samples have a high degree of crystallinity (>50%) throughout the wide range of compositions from 0 to 95 mol % 3HV (see Table I). Only two crystalline forms are detectable in Figure 1. The P(3HB) crystal lattice (fiber repeat 0.596 nm)[7,8] is observed for the samples with compositions from 0 to 37 mol % 3 HV, while the P(3HV) crystal lattice (fiber repeat 0.556 nm)[25,26] is observed for the samples with compositions from 53 to 95 mol % 3HV. The transformation from the P-(3HB) lattice to the P(3HV) lattice seems to occur at approximately 40 mol % 3HV. Bluhm et al.[18] have found the same lattice transition and the high level of crystallinity for the P(3HB-*co*-3HV) samples with compositions up to 47 mol % 3HV. Our result confirms their data.

The unit cells of P(3HB) and P(3HV) are orthorhombic, $P2_12_12_1$ (D_2^4) with a = 0.576 nm, b = 1.320 nm, and c = 0.596 nm (fiber repeat) for P(3HB)[7] and a = 0.952 nm, b = 1.008 nm, and c = 0.556 nm (fiber repeat) for P(3HV).[26] Two molecules of the bacterial polyesters pass through the unit cell. The d spacings from X-ray diffraction diagrams of P(3HB-*co*-3HV) samples are given in Table I. For the copolyester samples (3HV = 0–37 mol %) exhibiting the P(3HB) lattice pattern we calculated (020), (110), and (002) d spacings. The (110) d spacing apparently increases as the 3HV content increases to 37 mol %, while the (020) and (002) d spacings remain unchanged, indicating that only the a parameter of the unit cell changes. It is concluded that the ethyl side chains of 3HV units expand the (110) plane of the P(3HB) lattice due to the steric effects. For the copolyester samples (3HV = 53–95 mol %) exhibiting the P(3HV) lattice pattern, we calculated (020), (110), and (211) d spacings. As can be seen from Table I, there are no changes in the all-d spacings, leading to the conclusion that the parameters of the P(3HV) unit cell are not influenced by the methyl side chains of 3HB units.

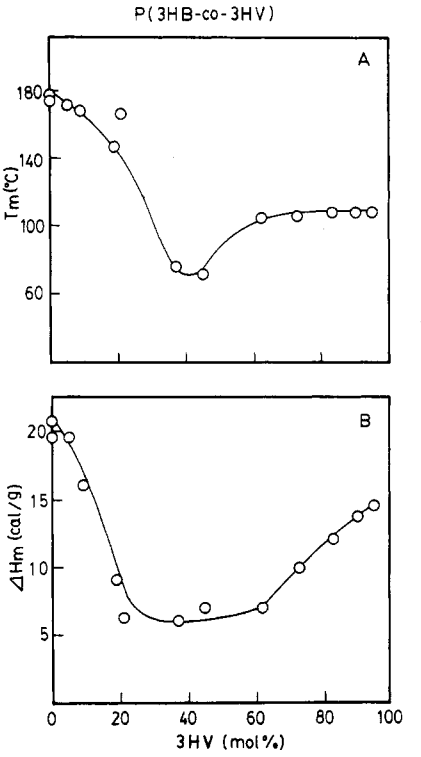

Figure 2. Melting temperature (T_m) and enthalpy of fusion (ΔH_m) versus composition curves for as-isolated P(3HB-*co*-3HV) samples. Samples were heated at 10 °C/min.

Figure 2 shows the melting temperatures (T_m) and the enthalpies of fusion (ΔH_m) for as-isolated P(3HB-*co*-3HV) samples in powder form. The T_m value of P(3HB) homopolymer was 178 °C, and the T_m value of P(3HB-*co*-95%3HV) was 108 °C. A minimum value (around 75 °C) of T_m was observed at approximately 40 mol % 3HV, where the crystal lattice transition took place. The enthalpy of fusion (ΔH_m) versus composition curve shows a similar trend with the T_m versus composition curve.

P(3HB-*co*-4HB). Figure 3 shows the X-ray diffraction patterns of solution-cast P(3HB-*co*-4HB) films containing from 0 to 49 mol % 4HB. Only one crystalline form of the P(3HB) lattice is observed for the copolyesters with compositions up to 49 mol % 4HB. However, the X-ray diffraction pattern of P(3HB-*co*-49%4HB) sample is almost featureless. The degree of X-ray crystallinity decreases from 55 to 14% as the 4HB content in the copolyester increases from 0 to 49 mol % (see Table II). This indicates that 4HB units cannot crystallize in the sequence of 3HB units and act as defects in the P(3HB) crystal lattice.

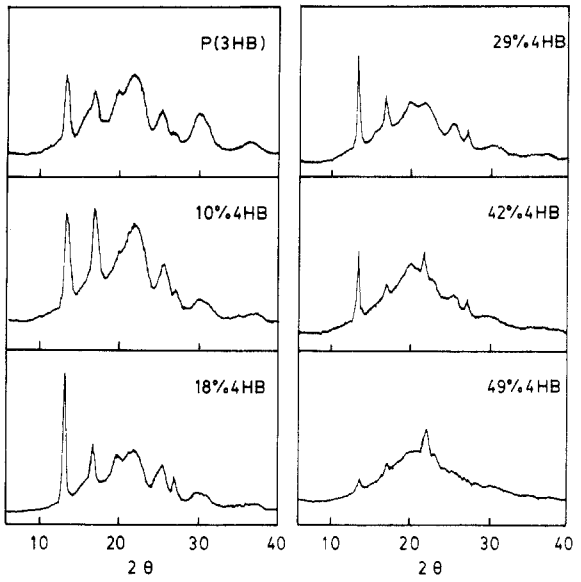

Figure 3. X-ray diffraction patterns of P(3HB) and P(3HB-*co*-4HB) films cast from $CHCl_3$ solution. Samples had been aged for 3 days at room temperature after evaporation of the solvent.

Table II
Degree of Crystallinity, Crystalline Structure, and *d* Spacings of P(3HB-*co*-4HB) Samples[a] from X-ray Diffraction

4HB, mol %	crystallinity, %	cryst struct	*d* spacings,[b] nm (020)	(110)	(002)
0	55 ± 5	P(3HB)	0.659	0.525	0.296
10	46 ± 5	P(3HB)	0.661	0.524	0.296
18	45 ± 5	P(3HB)	0.663	0.525	0.295
29	38 ± 5	P(3HB)	0.654	0.520	0.295
42	23 ± 5	P(3HB)	0.644	0.516	0.296
49	14 ± 5	P(3HB)	0.643	0.514	

[a] Solution-cast films at 3 days after evaporation of the solvent ($CHCl_3$). [b] The deviations in these values are ±0.005 nm.

The (020), (110), and (002) *d* spacings of the P(3HB-*co*-4HB) samples from the X-ray diffraction diagrams are given in Table II. The *d* spacings are almost invariant in the composition range from 0 to 18 mol % 4HB. In the composition range from 18 to 49 mol % 4HB, the (002) *d* spacing remains unchanged, while the (020) and (110) *d* spacings apparently decrease with increase of the 4HB content. This result indicates that the lateral parameters of *a* and *b* axes for the P(3HB) unit cell are slightly reduced as the 4HB content increases from 18 to 49 mol %, while the fiber repeat (*c* parameter) is unchanged.

Figure 4 shows the melting temperatures (T_m) and the enthalpies of fusion for as-isolated P(3HB-*co*-4HB) samples in powder form. The T_m value of samples decreases slightly from 178 to 150 °C as the 4HB content increases from 0 to 18 mol %. However, the T_m values are almost constant in the composition range from 18 to 49 mol % 4HB. The enthalpies of fusion (ΔH_m) of these copolyesters decrease monotonously with an increase in the 4HB content in the composition range of 0–49 mol %.

Let us discuss the observed melting temperatures of random copolyesters of 3HB and 4HB units, using the Flory equation for melting temperature depression:[27]

$$1/T_m - 1/T_m^\circ = -R \ln (X_B)/\Delta H_u \qquad (1)$$

where ΔH_u is the enthalpy of fusion per mole of repeating units, R is the universal gas constant, T_m is the melting temperature of copolymer, T_m° is the melting temperature of P(3HB), and X_B is the mole fraction of 4HB units in the copolymer. The value of ΔH_u derived from the plot

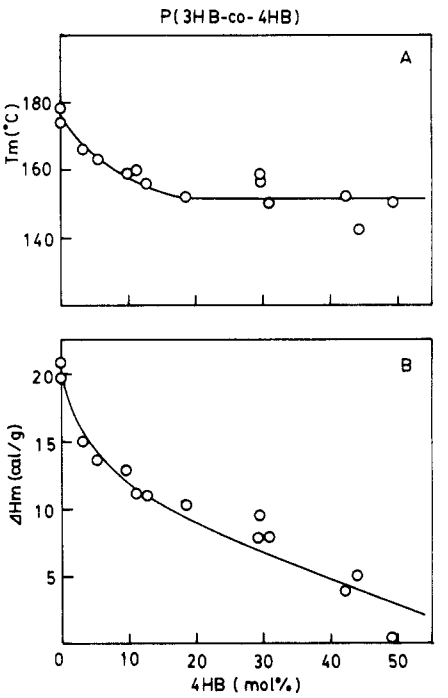

Figure 4. Melting temperature (T_m) and enthalpy of fusion (ΔH_m) versus composition curves for as-isolated P(3HB-*co*-4HB) samples. Samples were heated at 10 °C/min.

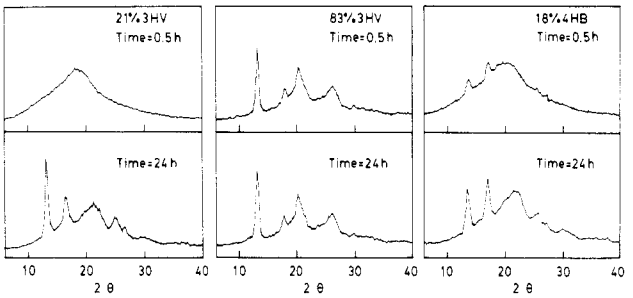

Figure 5. X-ray diffraction patterns of melt-quenched copolyester samples of P(3HB-*co*-21%3HV), P(3HB-*co*-83%3HV), and P-(3HB-*co*-18%4HB) after 0.5 and 24 h at room temperature.

of $1/T_m$ vs $-\ln X_B$ for the P(3HB-*co*-4HB) samples with compositions from 0 to 18 mol % 4HB was 2.88 kcal·mol^{-1}. This value is almost consistent with the observed value (3.05 kcal·mol^{-1}) of P(3HB) homopolymer corrected by the degree of X-ray crystallinity (55%). However, the observed T_m values of the copolyesters with compositions from 18 to 49 mol % 4HB deviated from the Flory equation (eq 1) The change in melting temperatures at around 20 mol % 4HB may correspond to the change in lateral parameters of *a* and *b* axes for the P(3HB) unit cell at around 20 mol % 4HB (see Table II).

Rate of Crystallization. The rate of crystallization was measured by X-ray diffraction for four samples of bacterial polyesters quenched from the melt. Figure 5 shows X-ray diffraction patterns of melt-quenched copolyester samples of P(3HB-*co*-21%3HV), P(3HB-*co*-83%3HV), and P-(3HB-*co*-18%4HB) after 0.5 and 24 h at room temperature. The melt-quenched sample of P(3HB-*co*-21%3HV) is still amorphous after 0.5 h, while the crystallization of melt-quenched P(3HB-*co*-83%3HV) sample is almost complete within 0.5 h. The melt-quenched sample of P(3HB-*co*-18%4HB) proceeds to crystallize at 0.5 h.

Figure 6 shows the time dependences of X-ray crystallinities for the melt-quenched samples of P(3HB) and three copolyesters. Both samples of P(3HB) and P(3HB-*co*-83%3HV) crystallize quickly at an identical rate. The rate

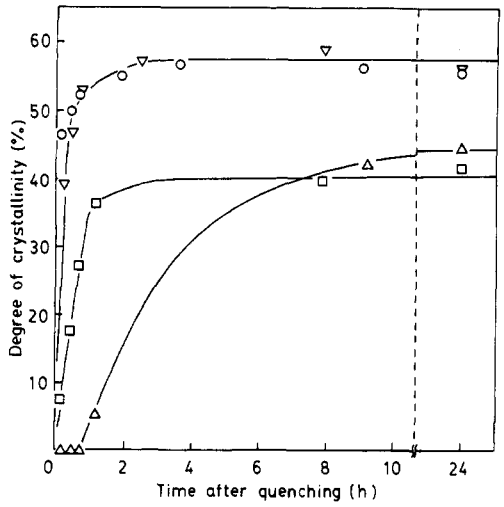

Figure 6. Time dependences of X-ray crystallinities for melt-quenched samples of P(3HB) (○), P(3HB-*co*-21%3HV) (△), P(3HB-*co*-83%3HV) (▽), and P(3HB-*co*-18%4HB) (□). The deviations in crystallinity are ±5%.

of crystallization of P(3HB-*co*-18%4HB) sample is slower than that of P(3HB) sample. The P(3HB-*co*-21%3HV) sample starts to crystallize after 0.5 h, and the rate of crystallization is much slower than that of P(3HB-*co*-18%4HB) sample. Thus, the rate of crystallization decreases in the following series:

$$\text{P(3HB)} \simeq \text{P(3HB-}co\text{-83\%3HV)} > \text{P(3HB-}co\text{-18\%4HB)} \gg \text{P(3HB-}co\text{-21\%3HV)} \quad (2)$$

The rate of crystallization is most likely to depend on both rates of nucleation and crystal growth. In the case of P(3HB-*co*-21%3HV) sample that crystallizes in the P(3HB) lattice, both the rates of nucleation and crystal growth are much slower than those of P(3HB) homopolymer. This indicates that the ethyl side chains of 3HV units prevent crystallization of P(3HB) lattice due to the steric effects. In contrast, both the rates of nucleation and crystal growth are not affected by the methyl side chains of 3HB units in the P(3HB-*co*-83%3HV) sample that crystallizes in the P(3HV) lattice. These composition effects on the rate of crystallization correspond to the results of X-ray diffraction d spacings that the (110) plane of the P(3HB) lattice expands as the content of 3HV units increases, while the unit cell parameters of the P(3HV) lattice are not influenced by the content of 3HB units. When 4HB units exist in the P(3HB) lattice, the nucleaion rate is apparently unaffected by 4HB units, but the rate of crystal growth slightly decreases.

In conclusion, the crystalline properties of bacterial copolyesters are strongly dependent on the compositions of monomeric units.

Registry No. (3HB)(3HV) (copolymer), 80181-31-3; (3HB)(4HB) (copolymer, 117068-64-1; P(3HB), 26063-00-3.

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