Microstructure of Bacterially Synthesized Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

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ABSTRACT: Bacterially synthesized copolyesters of 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV) of HV mole fraction ranging from 0 to 93% were analyzed by NMR spectroscopy and differential scanning calorimetry (DSC). The sequence distributions of some PHB-HV's, determined from ^{13}C NMR spectra, were statistically random (model 1), but those of the other samples were not. The sequence distributions of the latter obeyed the model of a mixture of two random copolymers (model 3). These samples had two or three peaks in DSC melting curves, indicating that they were mixtures of different polymers. The plots of melting points of the samples having two peaks in a melting curve against the HV mole fractions of two composed copolymers estimated from model 3 show the same tendency with those having a single peak, whose sequence distributions obey model 1, against HV mole fractions of the samples. Both results obtained by ^{13}C NMR spectra and DSC support the conclusion that some PHB-HV's are mixtures of random copolymers with different HV mole fractions.

Introduction

Poly(3-hydroxybutyrate) (PHB),[1] poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHB-HV),[2,3] and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)[4] were synthesized by microbial fermentation. The biosyntheses of these new polyesters have distinct differences from the usual processes for production of industrial polymers. In particular, the starting materials are not restricted to petroleum. The products are highly pure because no catalysts are used. The biosynthesized thermoplastic polyesters exhibit characteristic properties,[5-7] such as environmental degradability,[8,9] biocompatibility, optical activity, and so on. Some of these polyesters are now produced on a large scale as commercial thermoplastics and are suitable for medical applications such as a monofilament surgical suture and controlled drug release applications.

PHB-HV was first synthesized by Imperial Chemical Industries (ICI) with propionic acid and glucose as carbon sources,[2] but the HV mole fractions in ICI's PHB-HV's were limited to the range of 0 to 47 mol %. The impact strength, flexural modulus, and melting temperature of PHB-HV vary with the HV mole fraction.[10,11] PHB-HV was shown to have a statistically random copolymer sequence distribution of 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV).[11,12] These results were derived from the PHB-HV samples with 0 to 47 mol % HV contents. Recently, a series of PHB-HV with HV fractions of more than 47 mol % were biosynthesized by using valeric acid and butyric acid as carbon sources.[3] By this procedure, PHB-HV with the HV mole fractions of 0–95 mol % can be obtained. In this paper, we report the microstructures determined by NMR and DSC measurements for 15 biologically synthesized PHB-HV samples with various HV fractions ranging from 0 to 93 mol %. It will be shown that some of the PHB-HV samples are not simple random copolymers but mixtures of random copolymers of different HV mole fractions.

Experimental Section

Materials. Samples of PHB and PHB-HV were isolated from *Alcaligenes eutrophus* (ATCC17699, NCIB11599). The strain was maintained on nutrient agar slants at 4 °C by monthly subculture. The bacteria were first grown in 100 cm^3 of nutrient-rich medium containing 10 g of yeast extract, 10 g of polypeptone, 5 g of meat extract, and 5 g of $(NH_4)_2SO_4$ at 30 °C. After 24 h, these were harvested by centrifugation and washed with water. The accumulation of polyester was not observed in these bacteria at this stage. In order to start the biosynthesis of polyester, the washed bacteria were transferred into 100-cm^3 nitrogen-free mineral medium, pH 7, containing 2.0 g of carbon sources at 30 °C. After 48 h, these were harvested by centrifugation, washed with acetone and dried under vacuum at room temperature. Polyesters were extracted from dried cells with hot chloroform in a Soxhlet apparatus and purified by reprecipitation with hexane. A total of 1 PHB and 14 PHB-HV samples with different HV fractions was prepared from different carbon sources (see Table I).

Table I
Carbon Sources in the Fermentations

sample	carbon sources (amount, g)
1	glucose (2.0)
2	valeric acid (1.8) + butyric acid (0.2)
3	valeric acid (1.6) + butyric acid (0.4)
4	propionic acid (2.0)
5	propionic acid (2.0)
6	valeric acid (1.2) + butyric acid (0.8)
7	valeric acid (2.0)
8	valeric acid (2.0)
9	valeric acid (2.0)
10	valeric acid (2.0)
11	valeric acid (0.5) + butyric acid (1.5)
12	valeric acid (1.0) + butyric acid (1.0)
13	propionic acid (2.0)
14	valeric acid (1.4) + butyric acid (0.6)
15	propionic acid (2.0)

Mixtures of PHB and PHB-HV random copolymers were made by casting. Two selected polymers were weighed, dissolved in chloroform together, and cast at room temperature for 1 day and at 70 °C under vacuum for 1 day.

NMR Measurements. The 500-MHz ^1H NMR spectra were observed at 30 °C in $CDCl_3$ (10–15 g L^{-1}) on a JEOL GX-500 spectrometer with 6-s pulse repetition, 4000-Hz spectral width, 32K data points, and 16 accumulations. The ^1H noise decoupled 67.5-MHz ^{13}C NMR spectra were recorded at 30 °C in $CDCl_3$ (40–50 g L^{-1}) on a JEOL GSX-270 spectrometer with 5-s pulse repetition, 13 000-Hz spectral width, 64K data points, and 1500–2000 accumulations.

NMR Spectral Analysis. The relative peak intensities of ^{13}C NMR spectra were decided with a curve resolution program on

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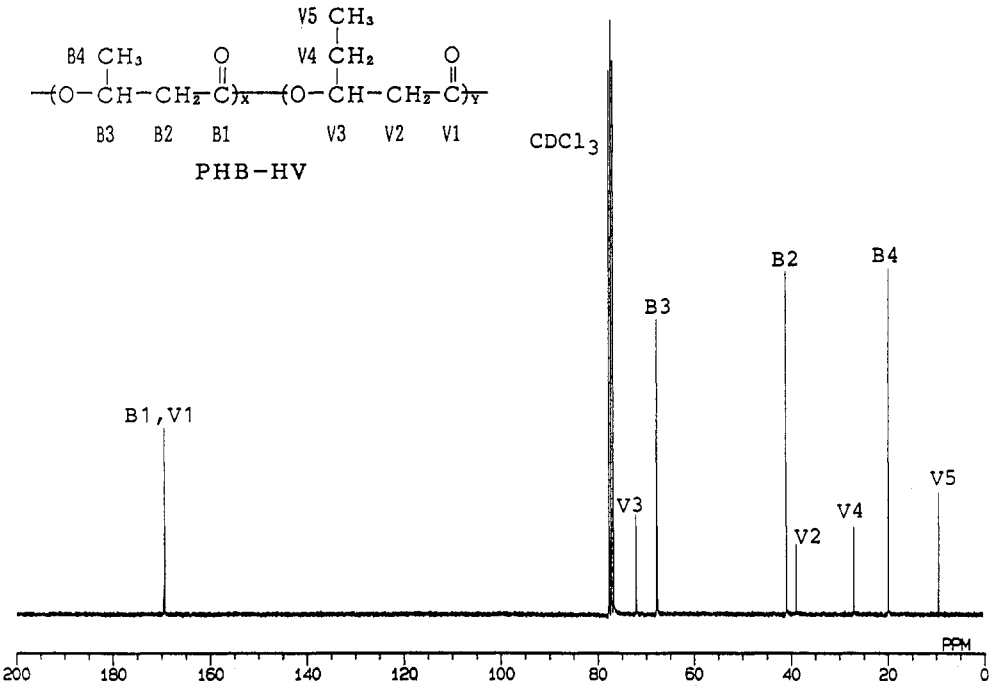

Figure 1. 67.5-MHz ^{13}C NMR spectrum of sample 6 in $CDCl_3$ at 30 °C.

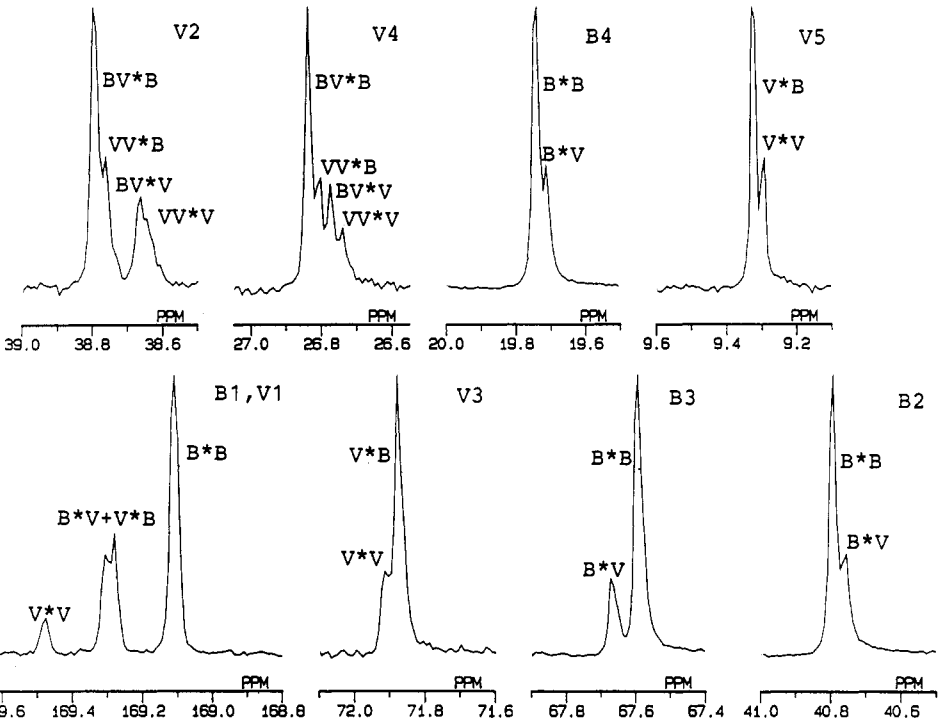

Figure 2. Splittings of individual resonances in the spectrum of Figure 1.

a minicomputer NEC PC-9801VM. With this program, a sum of Lorentzian curves of different peak sites and different peak intensities can be accurately obtained. The calculated spectra were visually matched to the experimental spectra to obtain an optimal fit. The optimized individual peak intensities are estimated from experimental chemical shifts, experimental total peak intensities, and line widths.

DSC Measurements. The thermal data were recorded on a SEIKO DSC-20 equipped with a thermal controller SSC-570. A heating rate of 20 °C min^{-1} was used for determination of the melting points. To avoid recrystallization, all samples for DSC measurement were heated to 200 °C, quenched in hexane at room temperature, and left for 5 days. The molecular weight of PHB is known to decrease rapidly at temperatures slightly above its melting point.[13-15] To avoid the influence of thermal degradation on DSC measurements, samples were newly prepared for each run.

Results and Discussion

NMR Analysis. The 67.5-MHz ^{13}C NMR spectra of sample 6 in $CDCl_3$ solution are shown in Figures 1 and 2. All the carbon resonances of PHB-HV are split into multiplets owing to diad and/or HV-centered triad comonomer sequences. The assignments of these signals have been reported.[11,12]

The relative peak intensities for the corresponding carbon resonances were estimated by the curve resolution program as described in the Experimental Section. The relative peak intensities so obtained are interpreted in terms of comonomer sequence distributions. The relative peak intensities obtained for the V2 and V4 resonances and the V1, B1, and B2 resonances were used to determine the HV-centered triad and diad sequence distributions,

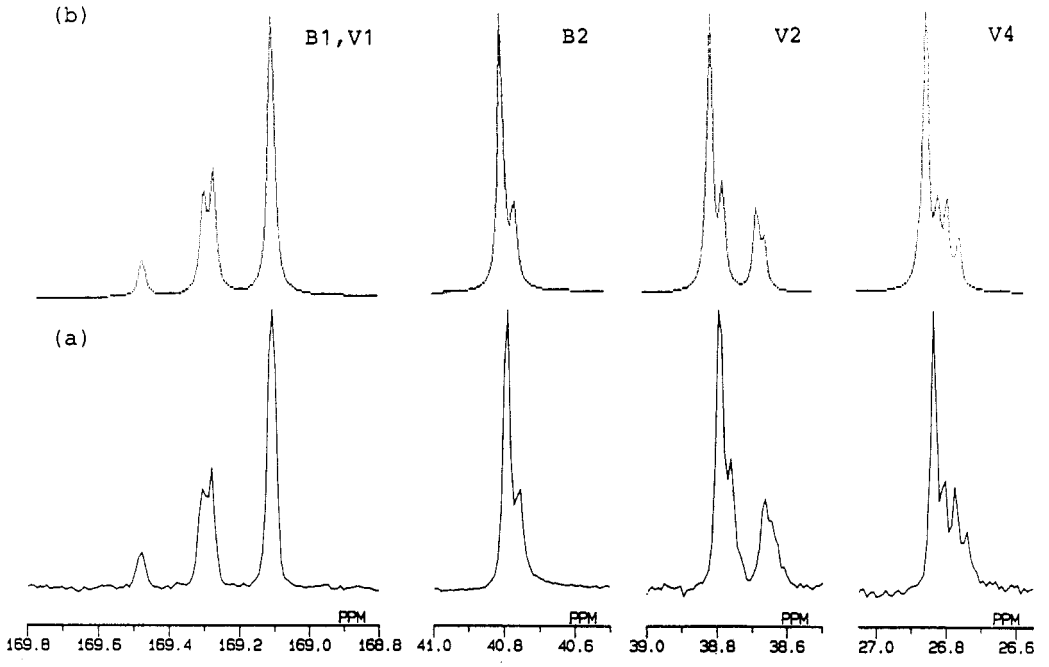

Figure 3. Experimental and calculated spectra of the B1, V1, B2, V2, and V4 carbon resonances of sample 6: (a) experimental; (b) calculated by the curve resolution program.

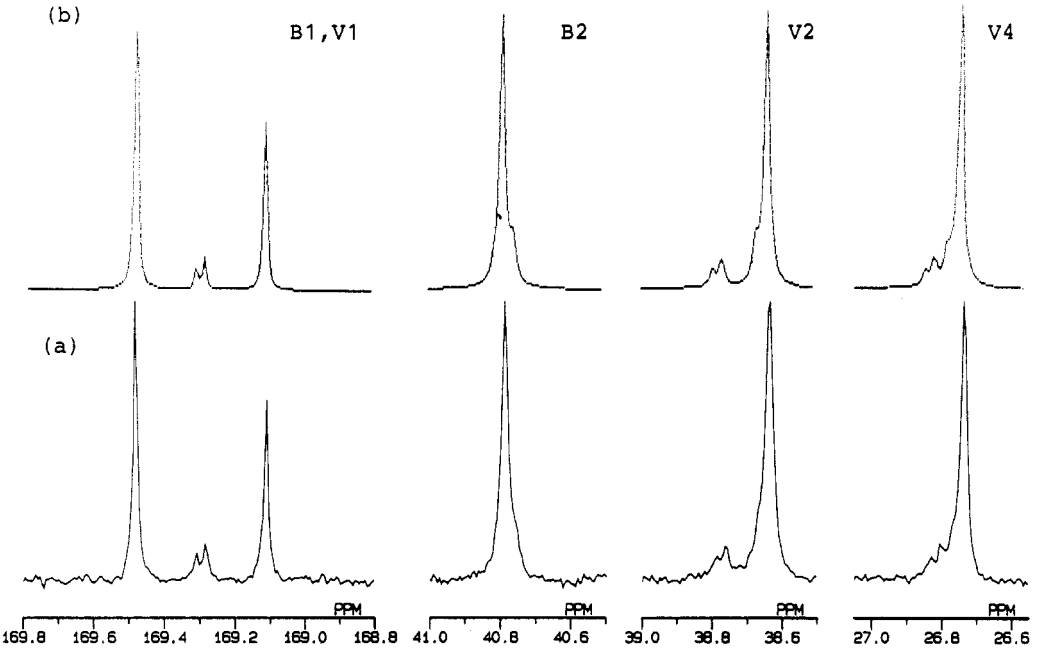

Figure 4. Experimental and calculated spectra of the B1, V1, B2, V2, and V4 carbon resonances of sample 7: (a) experimental; (b) calculated by the curve resolution program.

Table II
Experimental Diad and V-Centered Triad Relative Peak Intensities for Samples 6 and 7

sample		V	B	V*V	V*B	B*V	B*B	VV*V	BV*V	VV*B	BV*B
6	V1, B1	0.268	0.732	0.071	0.394		0.535				
	B2		0.773			0.165	0.608				
	V2	0.227		0.056	0.171			0.096	0.151	0.202	0.551
	V4							0.092	0.167	0.152	0.589
	av	0.248	0.752	0.064	0.184	0.181	0.571	0.024	0.040	0.044	0.140
7	V1, B1	0.601	0.399	0.546	0.110		0.344				
	B2		0.462			0.064	0.398				
	V2	0.538		0.474	0.064			0.801	0.081	0.073	0.045
	V4							0.834	0.060	0.061	0.045
	av	0.570	0.430	0.511	0.059	0.059	0.371	0.470	0.041	0.035	0.024

respectively. The spectra of samples 6 and 7 calculated with the optimized parameters, which were determined by the curve resolution method, are shown in Figures 3 and 4, respectively, together with the corresponding observed spectra. As shown in the figures, the agreement between the observed and the calculated spectra is good for both samples. Table II lists the relative peak intensities of samples 6 and 7 determined from the optimized parame-

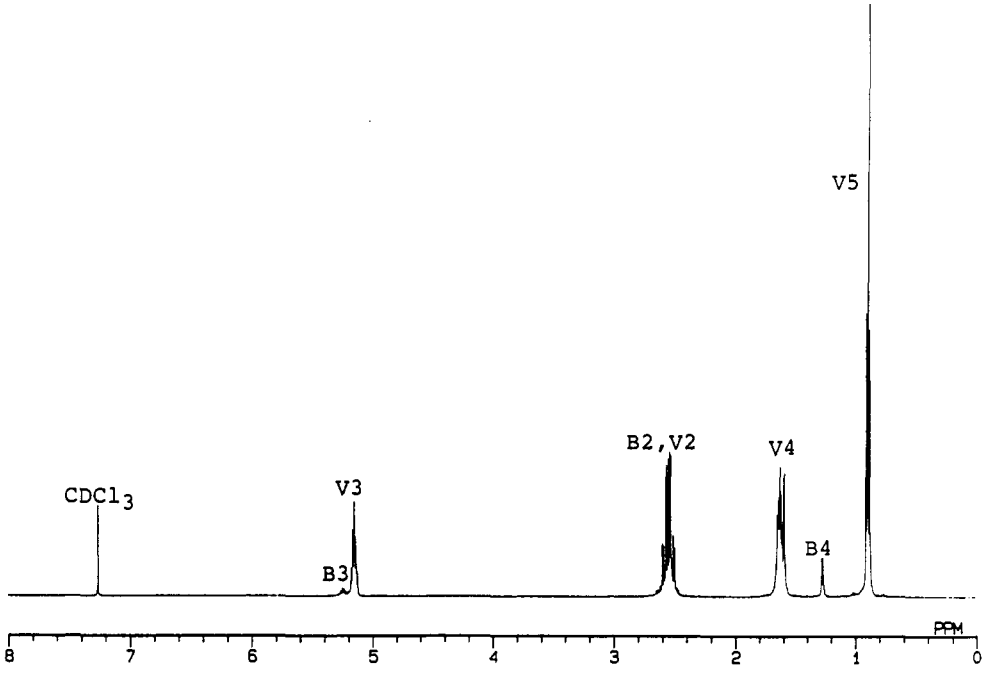

Figure 5. 500-MHz ^{1}H NMR spectrum of sample 9 in $CDCl_3$ at 30 °C.

ters. Similarly, the relative peak intensities, i.e., the sequence distributions, were determined for the other 12 PHB-HV samples. The mole, the diad, and the HV-centered triad fractions of HB and HV in the respective samples are summarized in Table III. For samples 9 and 11, it is difficult to estimate the sequence distributions accurately from ^{13}C NMR spectra because the resonances of the less abundant unit are too small to be analyzed. In the case of the former sample, HB resonances were practically not observed. In the latter case the signals of VV and VVV sequences were not observed. In these cases ^{1}H NMR spectra were used for the determination of HV mole fractions. The 500-MHz ^{1}H NMR spectrum of sample 9 in $CDCl_3$ is shown in Figure 5. The HV mole fractions of samples 9 and 11 were determined from the V5 and B4 proton relative peak intensities of the HV and HB units.[12,16] Those of samples 3 and 6–8 were also estimated in the same procedures. The results are listed in Table III. It has been reported that the sequence distributions of the PHB-HV are statistically random.[11,12] If this polymer is a statistically random copolymer describable by the Bernoullian statistics, F_{VV}, F_{VB}, F_{BV}, and F_{BB} (F_{XY} represents the mole fraction of XY sequence) can be expressed with the mole fraction of HV unit, F_V, as follows.

$$F_{VV} = F_V^2$$
$$F_{VB} = F_{BV} = F_V(1 - F_V)$$
$$F_{BB} = (1 - F_V)^2 \quad (1)$$

The diad-dependent carbonyl resonances in the ^{13}C NMR spectrum of sample 6 are split into four peaks with relative intensities described by eq 1 (see Figure 3), i.e., by a Bernoullian model. But in the case of sample 7 (see Figure 4), the signals of the B*V and V*B sequences are much smaller than those of the V*V and B*B sequences, indicating that the Bernoullian model cannot be applied to sample 7. In order to determine whether each sample is a random copolymer or not, a new parameter D is defined as follows.

$$D = \frac{F_{VV}F_{BB}}{F_{VB}F_{BV}} \quad (2)$$

It is clear from eq 1 that D is equal to 1 for a statistically random copolymer. The D values calculated for the samples are also given in Table III. Generally speaking, from eq 2, the D value for a "blocky" copolymer should be larger than 1 while that of an alternating copolymer should be smaller than 1. The D values of some samples in Table III are much larger than 1, indicating that those samples are of a block nature. The others appear to be random copolymers. No sample was found to have an alternating structure.

The blocky copolymer is considered to be either a block copolymer, a mixture of HV and HB homopolymers, or a mixture of HV- and HB-rich random copolymers. Considering that the PHB-HV was reported to be a random copolymer,[11,12] the blocky copolymer in Table III should be a mixture of random copolymers or a simple block copolymer.

We have calculated the sequence distributions according to the following three statistical models to characterize the microstructure of the samples investigated here. The first was the simplests random copolymer model, i.e., a Bernoullian model (model 1). In this model each diad and V-centered triad can be calculated from F_V^E as follows. (The parameters associated with the superscript E indicate the values that can be determined experimentally.)

$$F_{VV} = (F_V^E)^2 \quad F_{VB} = F_{BV} = F_V^E F_B^E \quad F_{BB} = (F_B^E)^2$$
$$F_{VVV} = (F_V^E)^3 \quad F_{BVV} = F_{VVB} = (F_V^E)^2 F_B^E$$
$$F_{BVB} = F_V^E (F_B^E)^2 \quad (3)$$

The second was a first-order Markovian model (model 2). In this case, the following relations can be derived.

$$P_{VV} = F_{VV}^E/F_V^E \quad P_{VB} = F_{VB}^E/F_V^E$$
$$P_{BV} = F_{BV}^E/F_B^E \quad P_{BB} = F_{BB}^E/F_B^E \quad (4)$$
$$F_{VV} = P_{VV}P_{BV}/(P_{VB} + P_{BV})$$
$$F_{VB} = F_{BV} = P_{VB}P_{BV}/(P_{VB} + P_{BV})$$
$$F_{BB} = P_{VB}P_{BB}/(P_{VB} + P_{BV})$$
$$F_{VVV} = P_{VV}^2P_{BV}/(P_{VB} + P_{BV})$$
$$F_{BVV} = F_{VVB} = P_{VV}P_{VB}P_{BV}/(P_{VB} + P_{BV})$$
$$F_{BVB} = P_{VB}^2P_{BV}/(P_{VB} + P_{BV}) \quad (5)$$

Table III
Experimental Sequence Distributions of PHB-HV Samples[a]

sample	F_V	F_V[b]	F_B	F_{VV}	F_{VB}	F_{BV}	F_{BB}	F_{VVV}	F_{BVV}	F_{VVB}	F_{BVB}	D[c]
1	0.000		1.000									
2	0.613		0.387	0.415	0.198	0.202	0.185	0.301	0.114	0.104	0.094	1.92
3	0.588	0.576	0.412	0.377	0.211	0.197	0.215	0.271	0.106	0.106	0.105	1.96
4	0.234		0.766	0.184	0.050	0.028	0.738	0.162	0.022	0.023	0.027	99.6
5	0.292		0.708	0.145	0.147	0.163	0.545	0.084	0.061	0.071	0.076	3.30
6	0.248	0.240	0.752	0.064	0.184	0.181	0.571	0.024	0.040	0.044	0.140	1.09
7	0.570	0.563	0.430	0.511	0.059	0.059	0.371	0.470	0.041	0.035	0.024	54.4
8	0.815	0.800	0.185	0.696	0.119	0.114	0.071	0.607	0.089	0.079	0.040	3.65
9[d]		0.925										
10	0.735		0.265	0.587	0.148	0.137	0.128	0.474	0.113	0.103	0.045	3.70
11	0.070	0.091	0.930	0[d]	0.070	0.081	0.849	0[d]	0[d]	0.007	0.063	
12	0.204		0.796	0.048	0.156	0.155	0.641	0.013	0.035	0.036	0.120	1.27
13	0.407		0.593	0.189	0.218	0.221	0.372	0.092	0.097	0.097	0.121	1.46
14	0.349		0.651	0.150	0.199	0.204	0.447	0.071	0.079	0.070	0.129	1.65
15	0.316		0.684	0.108	0.208	0.217	0.467	0.042	0.066	0.075	0.133	1.11

[a] F_X, F_{XY}, and F_{XYZ} indicate mole fractions of sequence X, XY, and XYZ, respectively, where X, Y, Z = V or B. [b] Decided from ^{1}H NMR spectra. [c] $D = F_{VV}F_{BB}/F_{VB}F_{BV}$. [d] Not determined from ^{13}C NMR spectra.

Here, P_{ij} (i,j = V or B) is conditional probability of j addition following the i ultimate unit at the propagating chain end. This model can be applied to block, random, and alternative copolymers and was used to examine the possibility of block copolymer. The last model was a mixture of two Bernoullian random copolymers (model 3). When the two Bernoullian model copolymers with the HV mole fractions of A and B are mixed with a molar ratio of X:$(1 - X)$, the values of A, B, and X can be calculated from the mole fractions of HV-centered triad sequences by solving the following equations using the Newton Method.

$$F_{VVV}^{E} = A^3X + B^3(1 - X)$$

$$F_{BVV}^{E} = F_{VVB}^{E} = A^2(1 - A)X + B^2(1 - B)(1 - X)$$

$$F_{BVB}^{E} = A(1 - A)^2X + B(1 - B)^2(1 - X) \qquad (6)$$

In this model, A, B, and X must converge in the range between 0 and 1 and then the mole, the diad, and the HV-centered triad fractions are calculated as follows.

$$F_V = AX + B(1 - X)$$

$$F_B = (1 - A)X + (1 - B)(1 - X)$$

$$F_{VV} = A^2X + B^2(1 - X)$$

$$F_{VB} = F_{BV} = A(1 - A)X + B(1 - B)(1 - X)$$

$$F_{BB} = (1 - A)^2X + (1 - B)^2(1 - X)$$

$$F_{VVV} = A^3X + B^3(1 - X)$$

$$F_{BVV} = F_{VVB} = A^2(1 - A)X + B^2(1 - B)(1 - X)$$

$$F_{BVB} = A(1 - A)^2X + B(1 - B)^2(1 - X) \qquad (7)$$

The calculated sequence distributions based on the above three models are given in Table IV. Samples 9 and 11 cannot be examined with models 2 and 3 because the diad and the HV-centered triad fractions cannot be obtained accurately. For the PHB-HV with the D value near 1, their observed sequence distributions were found to be completely interpretable on the basis of model 1 and therefore they should be random copolymers. On the other hand, for the PHB-HV's with the D value much larger than 1, the observed sequence distributions are consistent with the calculated distributions based on model 3, but not with model 2, so they are likely to be mixtures of random copolymers. With $0.67 < D < 1.5$, the sequence distributions follow model 1. These samples are simple random copolymers. The sample with $D > 1.5$ should be a mixture of two random copolymers. Considering that many other samples are reported to be random copolymers,[11,12] this conclusion appears to be reasonable.

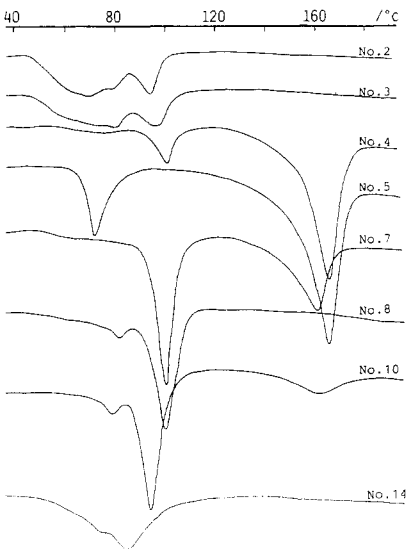

Figure 6. DSC melting curves for the samples with $D > 1.5$.

DSC Analysis. The melting points of the samples were estimated from the DSC melting curves. The samples with $D < 1.5$ have one peak in respective DSC melting curves, whereas the samples with $D > 1.5$ have two (samples 2–5, 7, 8, and 14) or three (sample 10) peaks. This result suggests that the samples with $D > 1.5$ are polymer blends. Samples 2–5, 7, 8, and 14, having two melting points, should be mixtures of two polymers, and sample 10, having three melting points, should be mixture of three polymers. The melting curves of the samples with $D > 1.5$ are shown in Figure 6. The melting points of the samples were taken as the tops of the DSC peaks. Table V lists the melting points of the samples investigated here.

The melting points of the four mixtures, composed of two selected polymers among the samples with $D < 1.5$ (random copolymers) and sample 1 (PHB homopolymer), were determined. In Figure 7 are plotted the melting point data against the mole fractions of Y polymers in X/Y mixtures. All the mixtures have two melting points except for the samples 1/sample 6 mixture. The samples 1/sample 6 mixture exhibits a single melting point corresponding to that of sample 1. This result indicates that only sample 1 crystallized and that sample 6 was amorphous. The melting points of the mixtures are independent of the mixing ratio of the samples used. Their melting

Table IV
Experimental and Calculated Sequence Distributions of PHB-HV Samples

sample	D	P_{VV}	P_{VB}	P_{BV}	P_{BB}	A	B	X	model	F_V	F_B	F_{VV}	F_{BV}	F_{BB}	F_{VVV}	F_{BVV}	F_{BVB}	SD[a]
2	1.92								exptl	0.61	0.39	0.42	0.20	0.18	0.30	0.11	0.09	B
									1	0.61	0.39	0.37	0.24	0.15	0.23	0.145	0.09	
		0.68	0.32	0.52	0.48				2	0.62	0.38	0.42	0.20	0.18	0.28	0.14	0.06	
						0.54	1.0	0.84	3	0.61	0.39	0.40	0.21	0.18	0.29	0.11	0.10	
3	1.96								exptl	0.59	0.41	0.38	0.20	0.22	0.27	0.11	0.10	B
									1	0.59	0.41	0.35	0.24	0.17	0.20	0.145	0.10	
		0.65	0.35	0.49	0.51				2	0.58	0.42	0.38	0.20	0.22	0.25	0.13	0.07	
						0.50	0.96	0.81	3	0.59	0.41	0.38	0.21	0.20	0.27	0.11	0.10	
4	99.6								exptl	0.23	0.77	0.18	0.04	0.74	0.16	0.02	0.03	B
									1	0.23	0.77	0.05	0.18	0.59	0.01	0.04	0.14	
		0.79	0.21	0.04	0.96				2	0.15	0.85	0.12	0.03	0.82	0.09	0.025	0.01	
						0.03	0.88	0.76	3	0.23	0.77	0.18	0.05	0.72	0.16	0.02	0.03	
5	3.30								exptl	0.29	0.71	0.15	0.15	0.55	0.08	0.07	0.07	B
									1	0.29	0.71	0.08	0.21	0.50	0.02	0.06	0.15	
		0.50	0.50	0.23	0.77				2	0.32	0.68	0.16	0.16	0.52	0.08	0.08	0.08	
						0.05	0.57	0.54	3	0.29	0.71	0.15	0.14	0.57	0.08	0.07	0.07	
6	1.09								exptl	0.25	0.75	0.07	0.18	0.57	0.03	0.04	0.14	R
									1	0.25	0.75	0.06	0.19	0.56	0.02	0.045	0.14	
		0.26	0.74	0.24	0.76				2	0.24	0.76	0.06	0.18	0.58	0.02	0.045	0.13	
						b	*b*	*b*	3									
7	54.4								exptl	0.57	0.43	0.51	0.06	0.37	0.47	0.04	0.02	B
									1	0.57	0.43	0.32	0.25	0.18	0.18	0.14	0.11	
		0.90	0.10	0.14	0.86				2	0.57	0.43	0.51	0.06	0.37	0.46	0.05	0.01	
						0.06	0.93	0.41	3	0.57	0.43	0.51	0.06	0.37	0.47	0.04	0.02	
8	3.65								exptl	0.81	0.19	0.70	0.115	0.07	0.61	0.08	0.04	B
									1	0.81	0.19	0.66	0.15	0.04	0.54	0.12	0.03	
		0.85	0.15	0.62	0.38				2	0.81	0.19	0.69	0.12	0.07	0.59	0.10	0.02	
						0.70	1.0	0.62	3	0.81	0.19	0.68	0.13	0.06	0.59	0.09	0.04	
9	*d*								exptl	0.93[c]	0.07[c]							
									1	0.93	0.07	0.86	0.07	0.00	0.79	0.07	0.00	
		d	*d*	*d*	*d*				2									
						d	*d*	*d*	3									
10	3.70								exptl	0.73	0.27	0.59	0.14	0.13	0.47	0.11	0.04	B
									1	0.74	0.26	0.54	0.195	0.07	0.40	0.145	0.05	
		0.80	0.20	0.52	0.48				2	0.72	0.28	0.58	0.145	0.13	0.46	0.115	0.03	
						0.40	0.83	0.21	3	0.73	0.27	0.57	0.165	0.10	0.46	0.11	0.05	
11	d								exptl	0.07	0.93		0.08	0.84		0.01	0.06	R
									1	0.07	0.93	0.00	0.07	0.86	0.00	0.00	0.07	
		d	*d*	0.09	0.91				2									
						d	*d*	*d*	3									
12	1.27								exptl	0.20	0.80	0.05	0.155	0.64	0.01	0.03	0.13	R
									1	0.20	0.80	0.04	0.16	0.64	0.01	0.03	0.13	
		0.24	0.76	0.19	0.81				2	0.20	0.80	0.05	0.155	0.64	0.01	0.035	0.12	
						0.15	0.32	0.67	3	0.20	0.80	0.05	0.155	0.64	0.01	0.035	0.12	
13	1.46								exptl	0.41	0.59	0.19	0.22	0.37	0.09	0.10	0.12	R
									1	0.41	0.59	0.17	0.24	0.35	0.07	0.10	0.14	
		0.46	0.54	0.37	0.63				2	0.41	0.59	0.19	0.22	0.37	0.09	0.10	0.12	
						0.15	0.49	0.25	3	0.41	0.59	0.19	0.22	0.37	0.09	0.10	0.12	
14	1.65								exptl	0.35	0.65	0.15	0.20	0.45	0.07	0.075	0.13	B
									1	0.35	0.65	0.12	0.23	0.42	0.04	0.08	0.15	
		0.43	0.57	0.31	0.69				2	0.35	0.65	0.15	0.20	0.45	0.07	0.085	0.11	
						0.29	0.65	0.84	3	0.35	0.65	0.14	0.21	0.44	0.07	0.07	0.14	
15	1.11								exptl	0.32	0.68	0.11	0.21	0.47	0.04	0.07	0.14	R
									1	0.32	0.68	0.10	0.22	0.46	0.03	0.07	0.15	
		0.36	0.64	0.33	0.67				2	0.34	0.66	0.12	0.22	0.44	0.04	0.08	0.14	
						0.12	0.39	0.27	3	0.31	0.69	0.12	0.20	0.48	0.04	0.07	0.13	

[a] Sequence distribution; R and B indicate random copolymer and blend of random copolymers, respectively. [b] Not converged in $0 < A, B, X < 1$. [c] Decided from ^{1}H NMR spectrum. [d] Cannot be estimated because the sequence distributions were not obtained accurately.

points correspond to those of the polymers composing the mixture.

The melting point of several PHB-HV samples were measured, and the results are shown in Figure 8. For the samples with $D < 1.5$, the melting points were plotted against the HV mole fractions of the polymer and, for the samples with $D > 1.5$, these were plotted against the HV mole fractions of the composed polymer, A and B, which were estimated by eq 6 and 7. The data for sample 10 were excluded from the plot because it shows three melting points. From eq 6 and 7, the HV mole fractions of the polymers can be determined only for the case of the mixtures of two polymers. All the plots, including those of the samples with $D > 1.5$ and having two melting points, fall on a simple curve, and this curve shows the same tendency as that of melting point vs HV mole fraction reported by Bluhm et al.[11] These results also support the conclusion that the samples with D values larger than 1.5

Table V
Melting Points of PHB-HV Samples

sample	D	sequence distribution	HV mole fraction[a] F_V	HV mole fraction[a] A or B	mp, °C
1		homo polymer	0.00		175.0
2	1.92	blend(2)		0.54, 1.00	69.4, 94.5
3	1.96	blend(2)		0.50, 0.96	74.0, 96.8
4	99.6	blend(2)		0.03, 0.88	166.6, 100.5
5	3.30	blend(2)		0.05, 0.57	166.8, 70.7
6	1.09	random copolymer	0.25		106.5
7	54.4	blend(2)		0.06, 0.93	162.0, 101.3
8	3.65	blend(2)		0.70, 1.00	82.3, 101.4
9		random copolymer	0.93		104.5
10	3.70	blend(3)			78.6, 95.6, 164.5
11		random copolymer	0.07		148.8
12	1.27	random copolymer	0.20		112.8
13	1.46	random copolymer	0.41		64.2
14	1.65	blend(2)		0.29, 0.65	84.4, 73.5
15	1.11	random copolymer	0.32		75.5

[a] F_V was estimated from ^{13}C NMR spectra; A or B was calculated with model 3. [b] Blend(2) and blend(3) indicate the mixtures of two and three random copolymers, respectively.

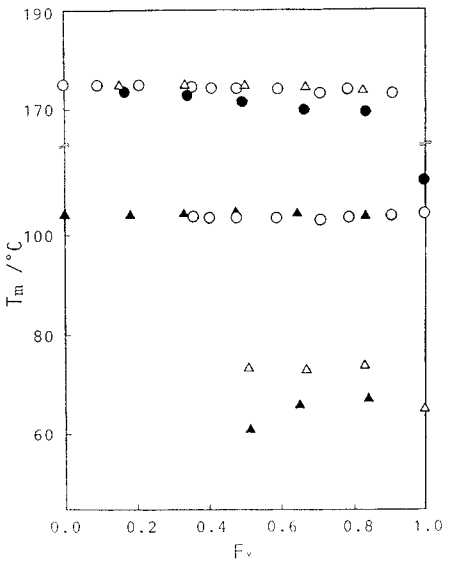

Figure 7. Plots of melting point (T_m) vs mole fraction of Y polymer (F_Y) in X/Y mixture: ●, sample 1/sample 6; △, sample 1/sample 13; ○, sample 1/sample 9; ▲, sample 9/sample 13.

are mixtures of the two random copolymers but not block copolymers.

Conclusion

In the bacterially synthesized copolyester of HB and HV, there are some copolymers whose sequence distributions follow neither the Bernoullian nor the first-order Markovian conditions, indicating that they are neither random nor block copolymers. These sequence distributions are

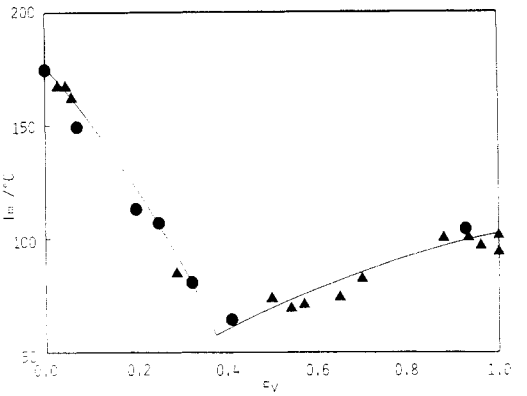

Figure 8. Melting points of bacterial PHB-HV's: ●, melting point vs HV mole fraction of the sample for a random copolymer with $D < 1.5$; ▲, two melting points vs calculated two HV mole fractions A and B from eq 6 and 7 for a sample with $D > 1.5$ presumed to be a mixture of two random copolymers.

interpretable on the basis of a model of a mixture of two random copolymers.

The unique PHB-HV samples exhibit two or three melting points corresponding to those of the individual random copolymers whose HV mole fractions are estimated on the basis of the model of the mixture of the random copolymers.

Both sequence distributions determined from NMR spectra and melting points from DSC melting curves support the conclusion that some of PHB-HV's are truly random copolymers and others are mixtures of random copolymers. A study of the biosynthesis conditions leading to the production of mixtures of random copolymers is in progress.

Registry No. (HB)(HV) (copolymer), 80181-31-3; glucose, 50-99-7; valeric acid, 109-52-4; butyric acid, 107-92-6; propionic acid, 79-09-4.

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