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Notes

Cocrystallization of Isothermally Crystallized Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)

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Introduction

Most random copolymers form crystals composed of the major comonomer units alone. Incorporation of the minor comonomer units into the crystalline phase costs a large amount of free energy. Thus, only a small amount of the minor units enters the crystals, where the minor units act as lattice defects.

On the other hand, a few copolymers are believed to form crystals comprising both monomer types.[1,2] When two types of monomer units have similar chemical structures, the excess free energy of cocrystllization is very small, and thus a significant amount of the minor monomer units is included in the crystalline phase. This phenomenon is called isomorphism.[3]

We have already reported experimental results[4] and a thermodynamic theory[5] of isomorphism. The latter provides a phase diagram for isomorphic copolymer systems. This diagram indicates that a melt and crystals coexist at a certain temperature range, where the composition in the melt phase is different from that in the crystalline phase. The content of the minor units in the crystalline phase increases with an increase in the whole copolymer and with a lowering of the melting (crystallization) temperature. The former feature has been consistent with the experimental data[4] for copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (P(3HB–3HV)) with various compositions (see next paragraph). The purpose of this paper is to confirm the latter feature experimentally using P(3HB–3HV)s.

The hypothesis of P(3HB–3HV) isodimorphism, i.e., inclusion of 3HV units in the P(3HB) crystalline lattice and of 3HB units in the P(3HV) lattice, was first proposed by Bluhm et al.[6] The degree of inclusion was, however, not determined. Recently, we have determined the comonomer compositions in the crystalline and amorphous phases by using solid-state high-resolution ^{13}C NMR spectroscopy[4] and estimated the degree of inclusion.

Barker et al.[7] have measured the densities of P(3HB–3HV)s containing up to 27 mol % HV and suggested that the rise of the crystallization temperature is accompanied by a decrease in the HV content in the crystalline phase. This is consistent with the expectation from the theoretical consideration.[5] The composition in the crystalline phase, however, was not determined. In the present paper, the partitioning of comonomer units between the crystalline and amorphous phases for P(3HB–3HV)s crystallized at various temperatures is determined by the technique reported previously.[4] The relationship between the crystallization temperature and the comonomer composition in the crystalline phase is analyzed.

Experimental Section

P(3HB–3HV) copolymers containing 18.3 and 55.4 mol % HV were used in this study. These comonomer compositions correspond to each side of the eutectic point.[6] P(3HB–3HV) containing 18.3 mol % HV (P(3HB–18.3% HV)) was purchased from Aldrich Chemical Co. P(3HB–55.4% 3HV) was isolated from *Alcaligenes eutrophus* (NCIB 11599). The details of bacterial preparation were described elsewhere.[8] The HV contents were determined from ^{1}H NMR spectra in chloroform solution.[6,9] Sequence distributions of the copolymers were confirmed to be random.[8]

After melting, the copolymers were quenched into a thermostated oven or iced water at the required crystallization temperature, left for 5 days, and left at room temperature for more than 5 days.

Solid-state high-resolution ^{13}C NMR spectra were recorded at 67.9 MHz on a JEOL GSX-270 spectrometer equipped with cross-polarization magic-angle sample spinning (CPMAS) accessories. All spectra were acquired with high-power dipolar decoupling (DD) of ca. 60 kHz and MAS at 5.0–5.5 kHz. CPMAS NMR spectra were measured with a 2-ms contact time, a 5-s pulse repetition, a 27-kHz spectral width, 8K data points, and 1000 accumulations. DDMAS NMR spectra were measured with a 45° pulse, a 5-s pulse repetition, a 27-kHz spectral width, 8K data points, and 1200–3600 accumulations. ^{13}C chemical shifts were calibrated indirectly by the methyl resonance of solid hexamethylbenzene (17.6 ppm relative to tetramethylsilane). The NMR 1 program supplied from New Methods Research Inc. was utilized for the curve resolution.

DSC thermal data were recovered on a Seiko DSC-20 equipped with a SSC-580 thermal controller. A heating rate of 20 °C min^{-1} was used.

Results

P(3HB–18.3% 3HV) and P(3HB–55.4% 3HV) were isothermally crystallized at temperatures in the range from 0 to 80 °C. The 67.9-MHz CPMAS NMR spectra of these samples were measured. The spectra are much similar to the spectra recorded previously for the same copolymers crystallized at room temperature.[4] It was reported that the chemical shifts of P(3HB–3HV)'s are dependent on their crystalline lattice.[4] The chemical shifts of the samples are independent of the crystallization temperature but dependent on the comonomer composition. Thus, P(3HB–18.3% 3HB) and P(3HB–55.4% 3HV) are crystallized in the P(3HB) and P(3HV) lattice, respectively. The lattice transition does not occur when the crystallization temperature is changed from 0 to 80 °C.

The 67.9-MHz ^{13}C DDMAS NMR spectra of isothermally crystallized P(3HB–3HV)s were also measured. In the DDMAS spectra measured under the conditions described in the Experimental Section, the peak intensities of the methyl resonances are proportional to the actual composition.[4] Each of the HB and HV methyl resonances was resolved into two peaks by using the NMR 1 program. The two peaks represent the contributions from the crystalline and amorphous phases, respectively. The

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Table I
Chemical Shift and Relative Peak Intensity of Methyl Resonances of DDMAS NMR Spectra of P(3HB–18.3% 3HV)s[a]

	chemical shift/ppm				relative peak intensity			
	HV unit		HB unit		HV unit		HB unit	
crystallizn temp/°C	cryst	amor	cryst	amor	amor	cryst	amor	cryst
0	9.95	10.81	20.08	21.27	0.173	0.047	0.208	0.571
20	9.95	10.74	20.20	21.05	0.129	0.066	0.267	0.538
40	9.96	11.08	20.22	21.07	0.132	0.052	0.156	0.660
60	9.89	10.90	20.16	20.99	0.144	0.047	0.186	0.623
80	9.94	10.88	20.15	21.00	0.115	0.058	0.079	0.748

[a] cryst and amor indicate the crystalline and amorphous phases, respectively.

Table II
Chemical Shift and Relative Peak Intensity of Methyl Resonances of DDMAS NMR Spectra of P(3HB–55.4% 3HV)s[a]

	chemical shift/ppm				relative peak intensity			
	HV unit		HB unit		HV unit		HB unit	
crystallizn temp/°C	amor	cryst	cryst	amor	amor	cryst	cryst	amor
0	10.59	11.01	19.87	20.69	0.304	0.245	0.168	0.283
20	10.42	11.03	19.94	21.13	0.320	0.221	0.135	0.324
40	10.54	11.13	19.97	21.13	0.179	0.404	0.183	0.234
60	10.51	11.15	20.03	21.22	0.255	0.325	0.209	0.211

[a] cryst and amor indicate the crystalline and amorphous phases, respectively.

Table III
Comonomer Composition (X[a]), Melting Point, and Crystallinity (λ) of P(3HB–18.3% 3HV)s Crystallized at Different Temperatures

crystallizn temp/°C	melting point/°C	heat of fusion/(J g^{-1})	X_c	X_a	X	λ
0	112.8	40.5	0.076	0.453	0.220	0.620
20	112.5	41.6	0.109	0.326	0.195	0.604
40	114.8	50.4	0.072	0.459	0.184	0.712
60	119.5	50.2	0.070	0.435	0.190	0.670
80	127.1	55.5	0.072	0.593	0.173	0.806

[a] X_c, X_a, and X indicate the HV content of the crystalline and amorphous phases and the whole copolymer, respectively, determined from solid-state ^{13}C DDMAS NMR spectra.

Table IV
Comonomer Composition,[a] Melting Point, and Crystallinity (λ) of P(3HB–55.4% 3HV)s Crystallized at Different Temperatures

crystallizn temp/°C	melting point/°C	heat of fusion/(J g^{-1})	X_c	X_a	X	λ
0	68.7	28.8	0.592	0.518	0.548	0.414
20	71.3	28.0	0.622	0.496	0.541	0.356
40	78.2	43.3	0.689	0.433	0.583	0.587
60	90.0 66.5	44.0	0.609	0.547	0.580	0.534

[a] See footnote a of Table III.

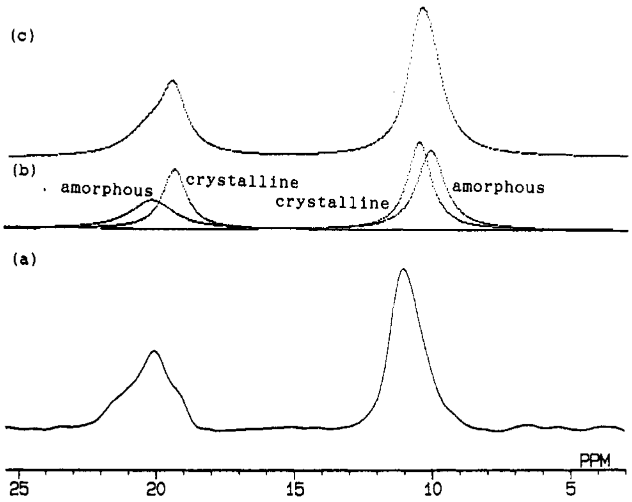

Figure 1. Methyl region of the 67.9-MHz ^{13}C DDMAS NMR spectrum of P(3HB–55.4% 3HV) crystallized at 0 °C: (a) observed spectrum; (b) simulated resonances for ^{13}C in the crystalline and amorphous phases; (c) total curve of b.

computer-fitted spectra of P(3HB–55.4% 3HV) crystallized at 0 °C are shown in Figure 1 together with the experimental spectrum. The assignments of the peaks were previously reported.[4] The chemical shifts and relative peak intensities of the crystalline and amorphous phases are listed in Tables I and II.

The HV contents in the crystalline (X_c) and amorphous (X_a) phases, together with the crystallinity (λ) and the HV content of the whole copolymer (X), are listed in Tables III and IV. These values were estimated from the relative peak intensities shown in Tables I and II. The values of X in Tables III and IV agree with the composition determined from ^{1}H NMR spectra in solution (0.183 or 0.554) within ±4 mol %. Thus, the experimental uncertainty of λ, X_c, and X_a should be within ±4 mol %. The melting point and heat of fusion determined from DSC thermograms are also listed in Tables III and IV. P(3HB–55.4% 3HV) crystallized at 60 °C has two melting peaks in its thermogram. The crystals having higher and lower melting points should form at 60 °C and at lower temperatures, respectively. X_c determined from the DDMAS spectrum is an average of mixed crystals and thus, is excluded from the following discussion.

Discussion

The rise in the crystallization temperature is accompanied by a decrease in the X_c of P(3HB–18.3% 3HV) and an increase in the X_c of P(3HB–55.4% 3HV), which is consistent with a theoretical consideration.[5] However, X_a changed independently of the crystallization temperature, which disagrees with the prediction of this theory.

Since the comonomer composition in the crystalline phase is different from that in the melt (amorphous phase), these compositions continuously change during the crystallization process. Thus, the crystalline phase forming at the earlier stage has a different comonomer composition from that forming at the later stage. The resulting solid copolymers should have a heterogeneous crystalline phase. The X_c determined from NMR spectroscopy is an average of the whole crystalline phase, whereas the observed X_a

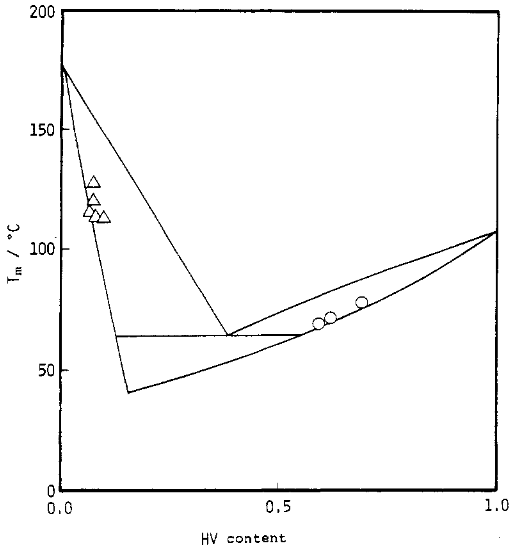

Figure 2. Relationship between the composition in the crystalline phase and the melting point of P(3HB–3HV) crystallized at various temperatures: (△) P(3HB–18.3% 3HV); (○) P(3HB–55.4% 3HV). The solid lines were theoretical curves calculated in the previous paper.[5]

is the composition in the amorphous phase after crystallization has terminated. If crystallization proceeds under thermodynamic conditions, the amorphous phase with the composition of the observed X_a is in equilibrium only with the crystalline phase forming at the latest period. Thus, the observed X_c is not in equilibrium with the observed X_a.

Since the crystalline phase of a copolymer is heterogeneous, the melting temperature is not observed as a single value but has a range of values. In this study, the average melting point (T_m) was determined from the peak of the DSC thermogram. The observed T_m should represent the melting point of the crystalline phase with composition X_c. The observed T_m is independent of the observed X_a.

The observed X_cs in P(3HB–18.3% 3HV) and P(3HB–55.4% 3HV) are plotted against the observed T_ms in Figure 2. The solid lines in this figure represent the calculated curve estimated in the previous paper.[5] The data of this study are well reproduced by the same calculated curve. Thus, P(3HB–3HV) copolymers, whose comonomer compositions in the crystalline phases are the same, should have the same melting point, even if the composition of the whole copolymer and/or the crystallization temperature are different from each other.

Conclusions

P(3HB–3HV)s crystallized at various temperatures were analyzed by solid-state high-resolution ^{13}C NMR spectroscopy. These copolymers have random sequence distributions. The crystalline lattices of these copolymers are independent of the crystallization temperature, whereas the composition in the crystalline phase changes. P(3HB–18.3% 3HV) and P(3HB–55.4% 3HV) are crystallized in the P(3HB) and P(3HV) lattices, respectively. The rise in the crystallization temperature is accompanied by the decrease in the content of minor comonomer units in the crystalline phase. The relationship between the composition in the crystalline phase and the melting point is consistent with the expectation from the phase diagram proposed in the previous paper.[5] Even if the composition of the whole copolymer and/or the crystallization temperature are different, the crystalline phases of P(3HB–3HV) copolymers having the same composition should have the same melting point.

References and Notes

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Registry No. P(3HB–3HV) (copolymer), 80181-31-3.