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AMINOLYSIS OF POLY (β -HYDROXYBUTYRATE) AND ITS COPOLYMER

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ABSTRACT: Poly (β -hydroxybutyrate) and its copolymer poly (β -hydroxybutyrate-co- β -hydroxyvalerate) P (HB-co-HV) were treated with methyl- and ethylamines. Melting peak temperatures, number-average molecular weights ($\bar{M}_n$) and long spacings (L) of treated samples decreased as the concentration of amines or the treating time increased. However, $\bar{M}_n$ and L almost levelled off at the optimum conditions, where amorphous regions at the surfaces of crystal lamellae were removed to isolate the core parts of single crystals. The level-off $\bar{M}_n$ values corresponding to the real thickness of the crystal core were estimated. The equilibrium melting point of PHB was ca. 200 °C which was estimated from the plot of melting point T_m against L^{-1} . The content of HV component in the crystalline region of P (HB-co-20 mol% HV) evaluated by $^1\text{H-NMR}$ was 18 mol%, which was slightly smaller than the initial composition (20 mol%). This implies that a small number of HV units was excluded from the HB crystals due to the bulky side group of the HV unit.

1. INTRODUCTION

Poly (β -hydroxybutyrate) (PHB) and its copolymer poly (β -hydroxybutyrate-co- β -hydroxyvalerate) P (HB-co-HV) are optically active aliphatic polyesters produced by various types of bacteria. PHB and P (HB-co-HV) are degradable either by enzymatic or hydrolytic degradation processes [1,2]. Thermal degradation of PHB and its copolymer has been reported by several authors [3-8]. PHB has been degraded by aqueous methylamine [9] or gaseous methylamine [10], where the fragment lengths equal to once and twice the value of the lamellar thickness have been found.

In the present study, continued from the previous report [9], PHB and P (HB-co-20 mol% HV) were treated with methyl- and ethylamines in order to remove the amorphous part at the crystal surface. This method has been widely applied to synthetic polyesters such as poly(ethylene terephthalate) [11]. The accordance of the observed long spacing with the crystal thickness estimated from the molecular weight was investigated. It is intended to find the optimum conditions of aminolysis, where the removal of the amorphous part at the crystal surfaces is completed,

in order to evaluate the real layer thickness of crystal and the content of HV component in the crystalline region of P (HB-co-20 mol% HV).

2. EXPERIMENTAL

2.1 Materials and Aminolysis

PHB and the copolymer P (HB-co-20 mol% HV) were bacterial plastics of natural origin (molecular weight $\bar{M}_n$, ca. 3.8×10^5) provided by Imperial Chemical Industries. The single crystal mats of PHB and P (HB-co-20 mol% HV) were obtained from a 0.01% solution of the polymer in propylene carbonate by isothermal crystallization at 90 °C for 24 h and at 40 °C for a week, respectively. Both samples (ca. 0.3 g) were treated at 25 °C in aqueous methyl- and ethylamine solutions (10 mL). After the treatment, the samples were filtered (or centrifuged), washed with ethanol and then dried.

2.2 Methods

2.2.1 DSC and SAXS Measurements

The melting behavior of samples (3 mg) was studied with a Perkin-Elmer Model DSC-2 differential scanning calorimeter (DSC) at a heating rate of 10 °C/min under nitrogen atmosphere. The melting peak temperature after being corrected for the thermal lag (or

heating rate dependence) was defined as the melting point T_m with accuracy within $\pm 0.25^\circ\text{C}$. The temperature scale was calibrated with high-purity standards.

Small-angle X-ray scattering (SAXS) patterns were recorded photographically using nickel filtered $\text{CuK}\alpha$ X-ray beam from a Rigaku-Denki RU-200 with a pinhole collimation system ($0.2 - 0.2\text{ mm}\phi$) at a specimen-to-film distance of 25 cm.

2.2.2 GPC Measurement

GPC chromatograms were recorded at 38°C with an HLC-802A high performance liquid chromatograph (Tosoh Co., Ltd.) equipped with a series of columns of 60 cm TSKgel G2000H, G3000H and 30 cm TSKgel GMHXL ($\times 2$) and an RI-8 differential refractometer. The eluent was chloroform (flow rate, 1 mL/min) and a sample concentration was ca. 10 mg/mL. The number-average molecular weight ($\bar{M}_n$) was calibrated using polyethylene glycol and polystyrene standards [8]. In this measurement, 6000 counts corresponded to the elution volume of 34 mL.

The calibration curve was standardized with PHB and P(HB-co-20 mol% HV) samples having six different $\bar{M}_n$ values (from 6,300 to 385,000) examined by GPC and a low-angle laser-light scattering (GPC-LALLS, Tosoh) system.

3. RESULTS AND DISCUSSION

3.1 Melting Peak Temperatures and $\bar{M}_n$ of PHB Samples Treated with Various Concentrations of Amines

Fig. 1 shows DSC curves of PHB single crystal mats treated for 24 h in various concentrations of aqueous methylamine (MA) and ethylamine (EA) solutions. The untreated PHB mat shows two endothermic peaks at 157 and 175°C . The melting peak at 157°C corresponds to the crystals formed by isothermal crystallization at 90°C , while the higher-temperature peak is caused by the reorganized crystals formed during the DSC heating process [12]. The latter peak steeply decreased in its area as the concentration of MA increased, and finally disappeared at the concentration of 25–30%. This implies that the reorganization during heating process hardly occurred because of the removal of amorphous regions at crystal surfaces. The temperature of the former peak remarkably

Fig. 1. DSC curves for PHB samples treated for 24 h in various concentrations of MA (a) EA (b) solutions. Dashed lines show the second run for each sample.

Fig. 2. Plots of melting peak temperatures for PHB treated with MA (○, for 24 h; △, for 48 h) and EA (●, for 24 h) solutions against the amine concentrations.

shifted to the lower temperature side at the concentration range of 15–30%. The dashed lines show the second runs of heating for the samples once heated up to 185°C then cooled to room temperature. The peaks also shifted to the lower temperature side, implying a steep drop in molecular weight by the aminolysis. Two melting peaks of PHB treated in the EA solution shifted at first to lower temperatures with increasing

Fig. 3. GPC chromatograms for PHB samples treated for 24 h with various concentrations of MA (a) and EA (b) solutions.

concentration (Fig. 1b) in a manner similar to the case of MA, followed by a very small shifting to lower temperatures above the concentration of 20%. In contrast, the second run curves show steeper decreases in peak temperatures and areas of melting peaks, implying that the crystallization became more difficult because of replacing the methyl end group to a larger one, i.e., ethyl group.

Fig. 2 shows plots of melting peak temperatures of the samples treated with MA against the concentration of amines. The steep drop in peak temperature is observed in the range of amine concentration from 15% to 30%. For comparison, melting peak temperatures of the samples treated with EA are also plotted in Fig. 2. The drop in peak temperature of PHB treated with MA is steeper than that with EA. It has been reported that the reaction rate for EA reduces to ca. 1/8 as compared with MA, because of the increment in the steric factor though the basicities of both amines are very close [13].

Fig. 3 shows GPC chromatograms of PHB treated for 24 h with various concentrations of MA (a) and EA (b). The $\bar{M}_n$ value of the control PHB slightly decreased to 2.46×10^5 due to the degradation during the isothermal crystallization, while that treated in 10% MA decreased to 8.21×10^4 accompanied by a pair of peaks corresponding to $\bar{M}_n$ of 2025 and 4390. It has been reported that the latter twin peaks corre-

Fig. 4. DSC curves of PHB samples treated with 20% MA for various periods of time at 25 °C. Dashed lines show the second run. The two curves at the bottom are DSC curves for PHB treated at 5 °C for 24 and 100 h, respectively.

sponded to the fragment lengths of once and twice the value of the lamellar thickness [9,10]. The former peak decreased in its area and finally disappeared at the concentration above 20%, while the twin peaks became more prominent with increasing concentration. The area of the peak at the higher count in the twin peaks gradually increased as the concentration increased, while that of the lower-count peak decreased. The $\bar{M}_n$ values estimated for each peak of the doublet slightly decreased to 1640 and 3170 for PHB treated in 40% MA. The depression in $\bar{M}_n$ for PHB treated in EA is smaller than that for MA, reflecting the milder treatment effect because of the larger steric factor [13].

3.2 Melting Peak Temperatures and $\bar{M}_n$ of PHB Treated with MA for Various Periods of Time

Fig. 4 shows DSC curves of PHB mats treated with 20% MA for various periods of time. The effect of treating time is almost the same as that of the amine concentration. The area of the melting peak at the

Fig. 5. GPC chromatograms for PHB samples treated with 20% MA for various periods of time at 25 and 5 °C.

lower temperature increased while that of the other peak decreased. At treating times above 48 h, the melting peak became single as a result of the disappearance of the peak at the higher temperature. The dashed lines show DSC curves of the second run. No melting peak was observed for PHB treated for longer than 48 h. This is explained by the fact that the recrystallization became more difficult because the amorphous regions at crystal surfaces were almost removed and the chain ends were replaced by methylamide and hydroxyl groups [11]. The melting peak temperatures of PHB treated in MA for 48 h plotted in Fig. 2 are slightly lower than those treated for 24 h, implying that the treatment is almost levelled off in this range of time.

Fig. 5 shows GPC chromatograms of the samples treated with 20% MA for various periods of time. The peak shifted stepwise to higher counts as the treating time increased. At 24 h, the sample shows only a pair of peaks corresponding to the fragment lengths of once and twice the lamellar crystal thick-

Fig. 6. DSC curves (a) and GPC chromatograms (b) for P(HB-co-20 mol% HV) samples treated with 20% MA solution for 24 and 48 h, respectively.

ness. With increasing treating time, the area of the peak at the higher count number increased while that of the other peak decreased, reflecting the progress in removal of amorphous regions. The changes in melting peak temperatures and $\bar{M}_n$ of PHB treated with EA were similar to those treated with MA.

PHB treated at a low temperature shows a less decrease in $\bar{M}_n$ with treating time. Two examples are given in Figs. 4 and 5, where melting peak temperatures and $\bar{M}_n$ of PHB treated in 20% methylamine at 5 °C for 24 and 100 h closely corresponded to those treated at 25 °C for 10 and 24 h, respectively.

3.3 Melting Peak Temperatures and $\bar{M}_n$ of P(HB-co-20 mol% HV) Sample Treated with Amines

Fig. 6 shows DSC curves (a) and GPC chromatograms (b) of P(HB-co-20 mol% HV) treated with 20% MA solution for 24 and 48 h. The comonomer sequence distribution of this copolymer was reported to be almost random [14]. The depression of melting peak temperatures is far smaller than that for

PHB, though the depression of $\bar{M}_n$ was similar to that for PHB. The copolymer treated for 48 h showed no melting peak at the second run of DSC. This may be due to the fact that crystalline parts distorted by inclusion of the HV units were easily attacked with the amines, while the extent of excess distortion of the HB crystal lattice caused by the aminolysis was relatively smaller than that of PHB.

3.4 Correspondence of Weight Loss to Nitrogen Content

Fig. 7a shows plots of weight loss of PHB and the copolymer treated in 20% MA against the treating time. The weight loss of PHB treated in 20% EA was

Fig. 7. Weight loss (a) and contents of C, H and N (b) of PHB and P(HB-co-20 mol% HV) samples treated in 20% MA as a function of treating time: (a) ○, PHB; ×, P(HB-co-20 mol% HV); (b) contents of C (○), N (□) and H (△) for PHB; contents of C (●), N (■) and H (▲) for P(HB-co-20 mol% HV).

slightly smaller than that in 20% MA. The weight loss of P(HB-co-20 mol% HV) is larger than that of PHB, reflecting the strong effect of crystallinity on the weight loss in a manner similar to that reported for poly(ethylene terephthalate) [15].

Fig. 7b shows plots of contents of C, H and N of PHB and P(HB-co-20 mol% HV) treated in 20% MA against the treating time. Theoretical contents of C and H are 55.8 and 7.0%, respectively, for PHB, and 56.8 and 7.2%, respectively, for the copolymer, to which the experimental values were very close. The C content decreased while the N content increased for both samples as the treating time increased. The N content of the copolymer increased faster than that of PHB, in a manner very similar to the weight loss curves. The curves of weight loss and N content almost levelled off at the treating time above 48 h, reflecting the almost complete removal of the folded chains at the crystal surfaces. The H content for both samples increased in a manner similar to that of N content as a result of the formation of end groups -CONHCH₃ or -OH after the molecular chain scission at the ester group, -CO-O- [11].

3.5 Estimation of Actual Crystal Thickness

Table 1 lists melting points (T_m), $\bar{M}_n$ and long spacings (L) of PHB and P(HB-co-20 mol% HV) treated in 20% MA solution. The $\bar{M}_n$ value of PHB crystallized at 90 °C was 2.46×10^5 . The $\bar{M}_n$ value decreased to 1885 and 4020, and to 1710 and 3635 for both samples treated for 24 and 48 h, respectively. PHB mats crystallized at 90 °C showed L of 6.65

Table 1 T_m , L and $\bar{M}_n$ of PHB and P(HB-co-20 mol% HV)

Sample	T_c (°C)	Time (h)	T_m (°C)	L (nm)	$\bar{M}_n \times 10^{-3}$	L_G (nm) ^{a)}
PHB	90	0	157	6.65	246	
	90	24	149.5	6.37	1.89;4.02	6.54;13.9
	90	48	147.5	6.16	1.71;3.63	5.93;12.6
	50	0	136	4.51	281	
	50	48	129	4.18	1.15;2.38	3.98;8.25
	110	0	168.5	9.35	184	
	110	24	158	8.46	2.85;5.66	9.87;19.6
	110	48	156	8.28	2.39;4.81	8.28;16.7
P(HB-co-20 mol% HV)	40	0	119.5	6.08	385	
	40	24	113	5.62	1.60;3.36	5.36;11.3
	40	48	108	5.07	1.32;2.84	4.42;9.53

^{a)} Lengths of fragments calculated for the pair of peaks in GPC chromatograms.

nm which decreased to 6.16 nm after the treatment for 48 h. The length calculated from $\bar{M}_n(L_G)$ of PHB treated for 48 h, using PHB crystal lattice parameters [16], is 5.93 nm. This value is very close to the L value of 6.16 nm. This implies that the actual thickness of crystal core was obtained under these conditions as a result of the removal of amorphous regions at crystal surfaces, whereas $\bar{M}_n$ of 3635 (or L_G of 12.60 nm) approximately corresponds to twice this thickness, indicating that about a half of folded chains were cut. P(HB-co-20 mol% HV) mats showed L of 6.08 nm and $\bar{M}_n$ of 3.85×10^5 , which decreased to 5.07 nm and 1315, respectively, after the treatment for 48 h. The L_G value of the copolymer treated for 48 h was considerably smaller than the value of L . This suggests that crystalline parts of the copolymer distorted by inclusion of the HV units were attacked by the amine under the conditions. For comparison, the same aminolysis method was applied to single crystal mats of PHB crystallized at 50 and 110 °C. The resultant T_m , L and $\bar{M}_n$ are also shown in Table 1. The L values for both mats were 4.51 and 9.35 nm, which decreased to 4.18 and 8.28 nm, $\bar{M}_n$ values were 1145 and 2390, and L_G values were 3.98 and 8.28 nm, respectively, after the treatment for 48 h. A close agreement was found between L and L_G of the treated samples. The L values for both samples almost levelled off by the treatment in 20% MA for the time above ca. 48 h. Therefore, these seem to be the optimum conditions for estimating the actual thickness of crystal core with no excess degradation of molecular chains in crystalline regions. In an ideal case, T_m corresponding to this thickness should be close to T_m of the untreated sample, although the actual T_m of the treated sample was ca. 10 °C lower than T_m of the untreated one probably because of the introduction of irregularity in chain arrangement into the surface regions of the crystal. The equilibrium melting point, obtained from the plot of T_m for untreated PHB mats against $1/L$ of corresponding mats treated for 48 h, was 200 °C which is 3 °C higher than the reported value [17,18].

3.6 Evaluation of HV Content in Crystalline Region of P (HB-co-20 mol% HV)

The content of HV component of P(HB-co-20 mol% HV) treated with 20% MA for 48 h estimated by ^1H -

NMR was 18 mol%, which is smaller than the initial composition of 20 mol% HV (evaluated by ^1H -NMR), but is considered to reflect the real HV content in the crystalline core. This result implies that a small number of HV units in copolymer chains was excluded from the HB crystal lattice during crystallization due to the presence of bulky side groups in the HV units. Kamiya et al. [19] have reported that a large number of HV units in the copolymers estimated by the solid-state ^{13}C -NMR are excluded from the HB crystal lattice.

4. CONCLUSIONS

Solution-grown single crystals of PHB and P(HB-co-20 mol% HV) treated with aqueous methyl- or ethylamine solution yielded the fragments with lengths corresponding to integer multiples (mainly one and two times) of crystal thickness. The aminolysis preferentially took place in the amorphous regions, so that the reaction in the copolymer was faster than in PHB because of the decrease in crystallinity from 81% (for PHB) to 53% (for the copolymer) [20]. The values of N content or weight loss almost levelled off under the optimum conditions, where the removal of amorphous regions at the crystal surfaces was completed. The treatment conditions, in 20% MA for ca. 48 h, were found to be optimum, where the L value of the treated sample was very close to the L_G value calculated from $\bar{M}_n$. This L value is considered to be close to the real crystal thickness. The equilibrium melting point of PHB was ca. 200 °C. The content of HV units in the crystalline region of P(HB-co-20 mol% HV) was 18 mol%. This suggests that a small number of HV units was excluded from the HB crystals due to the bulky side group of the HV unit.

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