Polymer Blends of Natural Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and a Synthetic Atactic Poly(3-hydroxybutyrate). Characterization and Biodegradation Studies

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ABSTRACT: Blends of synthetic atactic poly(3-hydroxybutyrate) (a-PHB) with a natural bacterial isotactic copolymer of 3-hydroxybutyrate with 3-hydroxyvalerate (PHBV) containing 10 mol % of 3HV units were prepared using a simple casting procedure. In the range of compositions explored (10−50% a-PHB), blends of bacterial PHBV and synthetic atactic a-PHB were miscible in the melt and solidified with spherulitic morphology. The influence of a-PHB content on the thermal and mechanical properties of the blends was evaluated. The degree of crystallinity decreased with increasing content of a-PHB in the film samples, and the elongation at break for a sample containing 50% of a-PHB was 30-fold that of pure PHBV. Degradation experiments, both hydrolytic (pH = 7.4, T = 70 °C) and enzymatic (PHB-depolymerase A from *Pseudomonas lemoignei*, Tris-HCl buffer (pH = 8), T = 37 °C), were performed for both polymers and polymer blends. The rate of enzymatic degradation of the blends was higher than that of PHBV and increased with a-PHB content in the blends studied, whereas pure a-PHB did not biodegrade under these conditions. 3-Hydroxybutyric acid and its dimer were identified by HPLC as biodegradation products of both pure PHBV and its blends with a-PHB. Higher oligomers up to heptamer were detected as degradation products of the blends by APCI-MS and ESI-MS.

Introduction

Poly(hydroxyalkanoate)s (PHA) are natural polyesters produced by microorganisms as carbon and energy reserves. Various PHA polymers can be produced depending on the bacterial strain and the cultivation conditions, their properties being controlled by their chemical structure and composition.[1] Industrial production of PHA by fermentation is rather expensive; thus alternative synthetic routes have been elaborated. Poly(β-butyrolactone), the synthetic analogue of natural PHB produced by bacteria, can be prepared by ring-opening polymerization of β-butyrolactone (β-BL) using various types of catalysts. The polymerization of β-BL by coordinative catalysts proceeds at high temperatures (50−100 °C) and yields PHB with a broad molecular weight distribution.[2−4] Anionic polymerization of β-BL using alkali metal alkoxide/crown ether initiator was found to be an effective method for atactic poly(3-hydroxybutyrate) (a-PHB) synthesis, yielding at room temperature polymers possessing number-average molecular weight (M_n) as high as 40 000 and narrow molecular weight distribution.[5,6] It was recently demonstrated that PHB samples with various tacticities (atactic, isotactic, and predominantly syndiotactic) can be obtained using this anionic catalyst.[7,8]

In order to improve mechanical properties and reduce the cost, blending of natural PHB with synthetic polymers was also performed.[9,10] Blends containing natural PHB and its synthetic analogues prepared with zinc- or tin-based initiators were recently reported,[11−14] and their biodegradability in the presence of PHB-depolymerase from *Pseudomonas pickettii* was investigated.[14] However, blends of synthetic atactic PHB with PHBV have not been investigated till now.

In this paper, miscibility studies of bacterial poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV, 10 mol % of 3HV units) with synthetic atactic poly(3-hydroxybutyrate) (a-PHB) prepared by alkali metal alkoxide initiators are presented. Physical property characterization and degradation behavior of the resulting blends, both by hydrolytic degradation and enzymatically by PHB-depolymerase A from *Pseudomonas lemoignei*, are reported.

Experimental Section

Materials. Bacterial PHBV (3HV content 10 mol %, M_n = 96 000, M_w/M_n = 2.2 by GPC) was supplied by Marlborough Biopolymers Ltd. (England). Atactic PHB (a-PHB; M_n = 38000; M_w/M_n = 1.14) was synthesized as described previously.[6] PHBV/a-PHB blends (90/10, 80/20, 50/50 wt %) were prepared by casting from chloroform solutions on glass plates at room temperature; films with a thickness of 0.2−0.3 mm were obtained.

Analytical Procedures. NMR spectra were recorded using a Varian VXR-300 multinuclear spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were run in $CDCl_3$ using TMS as an internal standard.

GPC experiments were conducted in THF solution at 35 °C at a flow rate of 1 mL/min using a Spectra-Physics 8800 solvent delivery system with two Mixed C Styragel columns in series and a Shodex SE 61 refractive index detector. Polystyrene standards with a low polydispersity (PL Lab.) were used to generate the calibration curve. A volume of 10 μL of sample solutions in $CHCl_3$ (concentration 2% w/v) was injected.

HPLC analyses were performed according to the procedure described by Abe et al.[14] using a HPLC Merck Hitachi L-4500

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A apparatus, with a gradient solvent delivery system and a diode array detector on a LiChrospher RP-8 (5 μm) stainless steel column (250 × 4 mm) at 40 °C. The gradient of distilled water (pH 2.5 maintained by the addition of HCl solution) to acetonitrile was adjusted for 40 min with a pump speed of 1.0 mL/min. Solutions containing products liberated during the enzymatic degradation were acidified to pH 2.5 with HCl solution, and 50 μL aliquots of sample solutions were injected into the HPLC system. Retention volumes, mass spectra, and UV spectra of degradation products in comparison with those of original standards of 3-hydroxybutyric acid and its dimer were determined (the a-PHB dimer standard was prepared via equimolar reaction of the 18-crown-6 complex of 3-hydroxybutyric acid potassium salt with β-butyrolactone, carried out in THF and terminated by protonation: ^{1}H NMR (D_2O) 1.21 (d, 3H), 1.30 (d, 3H), 2.45–2.66 (m, 4H), 4.22 (m, 1H), 5.26 (m, 1H)).

GC–MS analysis of products obtained by acidic methanolysis of PHBV was performed on a 30-m-long fused-silica DB-1701 capillary column, using a Varian 3300 gas chromatograph equipped with a Finnigan MAT 800AT ion trap detector.

Products of enzymatic degradation of PHBV/a-PHB blends were identified using a Finnigan MAT TSQ 700 mass spectrometer with respective APCI (atmospheric pressure chemical ionization) and ESI (electrospray ionization) interfaces. Sample solutions collected after a specified time of enzymatic degradation were introduced directly into the source via a Rheodyne injection valve.

FTIR spectra were recorded with an fts 40A Bio-Rad spectrometer at room temperature.

Differential scanning calorimetry (DSC) was performed using a Du Pont 9900 thermal analyzer in the temperature range −80 to +200 °C. The temperature scale was calibrated with high purity (>99.9%) standards (indium, benzene, *n*-octane).

Mechanical properties of the films were measured using an Instron Model 4204 tensile tester.

Wide-angle X-ray diffraction measurements (WAXS) were carried out with a Philips PW 1050/81 diffractometer controlled by a PW 1710 unit, using nickel-filtered Cu Kα radiation ($\lambda = 0.1542$ nm, 40 kV, 30 mA).

Isothermal crystallization experiments were performed using a Zeiss Axioscop optical polarizing microscope equipped with a Linkham TH600 hot stage. A video camera, attached to the microscope through the Linkham VTO232 interface, allowed real-time measurement of the spherulite dimensions on a TV monitor, after calibration with a micrometric reticule. Samples of PHBV and of blends containing 10 and 20% a-PHB were melted at 195 °C, rapidly quenched (cooling rate >250 °C/min) to the selected crystallization temperature (T_c), and then isothermally crystallized. This procedure led to a very high nucleation density in the case of the 50/50 blend. Therefore this blend was melted for 1 min at 200 °C to reduce nucleation. In order to minimize thermal degradation effects, a new sample was used for each crystallization measurement.

Enzymatic Degradation. Samples (films 25 × 8 mm^2) were incubated at 37 ± 0.1 °C in vials containing 1.5 mL of 50 mM Tris-HCl buffer, pH 8.0, 1 mM $CaCl_2$, in the presence of *P. lemoignei* PHB-depolymerase A (2.8 μg/mL) isolated and purified as described previously.[15] After 20 h the samples were removed from the solutions, washed with distilled water, dried under vacuum at room temperature for 4 h (to constant weight), and weighed on an analytical electronic balance (Sartorius RC210D; reproducibility ±0.02 mg). This procedure was repeated using fresh enzymatic solution for each 20 h incubation interval. As previously reported,[15] no appreciable decrease of enzyme activity was found after 20 h at 37 °C. The extent of biodegradation was quantified as weight loss divided by initial sample surface area ($\Delta m/S$). It was not possible to obtain a self-supporting film from pure a-PHB; therefore a solution of the polymer in CH_2Cl_2 was cast on a polypropylene thin sheet (25 × 8 mm^2). After solvent evaporation and drying under vacuum at room temperature to constant weight, the supported a-PHB film was subjected to biodegradation experiments. In this case, the exposed sample area was one half that of the self-supporting films of PHBV and blends. The water-soluble degradation products were analyzed by HPLC, APCI-MS, and ESI-MS techniques (see Analytical Procedures).

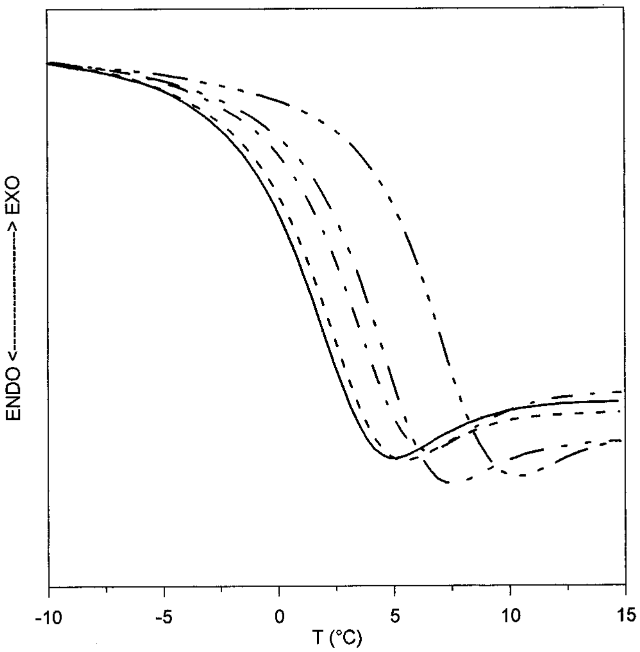

Figure 1. Calorimetric curves of PHBV (—), a-PHB (—•••—), and PHBV/a-PHB blends: 90/10 (---); 80/20 (—•—); 50/50 (—••—); heating rate 20 °C/min.

Hydrolytic Degradation. Samples of films prepared in the form of disks (12 mm diameter) from PHBV and PHBV/a-PHB blends were subjected to hydrolytic degradation in phosphate buffer (pH = 7.4) at 70 °C. Samples of a-PHB for hydrolytic degradation were prepared as described above for the enzymatic tests. After specified periods of degradation, the samples were separated from the solution, washed with distilled water, dried under vacuum, and analyzed by GPC and NMR.

Results and Discussion

Properties of Blends. The natural PHBV copolymer containing 10 mol % of 3-hydroxyvalerate units (HV) was used for preparation of blends with a-PHB. Its composition, estimated by ^{1}H NMR, was in good agreement with that determined by GC–MS analysis of the corresponding methyl esters (methyl 3-hydroxybutyrate, 89 mol %; methyl 3-hydroxyvalerate, 11 mol %) formed after acidic methanolysis of this copolymer, carried out as described in ref 2.

The poly(3-hydroxybutyrate) synthesized by anionic polymerization of β-butyrolactone in the presence of potassium methoxide/18-crown-6 complex according to ref 6 was found to be atactic and amorphous, as revealed respectively by NMR spectroscopy[8] and by DSC analysis.

The triad stereosequence distribution estimated by ^{13}C NMR from the methylene carbon region was equal to $I = 24\%$, $S = 25\%$, $H_s = 25\%$, $H_i = 26\%$. The content of isotactic diad fraction determined by ^{1}H NMR from the integration of the CH_3 peaks for the isotactic and syndiotactic diads was equal to 50%.

DSC curves in the glass transition range of melt-quenched PHBV/a-PHB blends and of the pure components (PHBV and a-PHB) are shown in Figure 1. Although the pure polymers have rather close glass transition temperatures (PHBV $T_g = 0$ °C, a-PHB $T_g = 6$ °C), in the blends an intermediate glass transition was found, whose T_g gradually changed with composition. All PHBV/a-PHB blends investigated showed melting

Table 1. Thermal and Mechanical Properties of PHBV and PHBV/a-PHB Blends

a-PHB (%)	T_m (°C)	$\Delta H_m{}^a$ (J/g)	$\Delta H_m(\mathrm{PHBV})^b$ (J/g)	$X_c{}^c$ (%) (WAXS)	$X_c{}^d$ (%) (DSC)	$\sigma_b{}^e$ (MPa)	$\epsilon_b{}^f$ (%)	E^g (MPa)
0	145	67	67	61	61	27	1 ± 1	1500
10	144	61	68	54	56	22	2 ± 1	940
20	142	55	69	51	50	20	3 ± 2	690
50	133	33	66	36	30	7	29 ± 5	240

[a] Melting enthalpy per gram of whole sample. [b] Melting enthalpy per gram of PHBV. [c] Crystallinity degree by WAXS (±5%). [d] Crystallinity degree by DSC (±2%). [e] Tensile strength. [f] Elongation at break. [g] Tensile modulus.

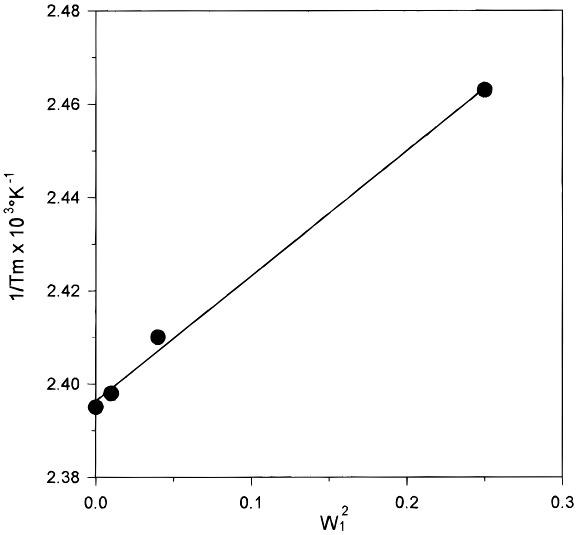

Figure 2. Melting temperature (T_m) of PHBV/a-PHB blends plotted according to eq 1; see text.

endotherms whose T_m temperatures and associated melting enthalpies (ΔH_m) decreased with increasing content of atactic component (Table 1).

In miscible blends containing a crystallizable polymer, the melting point depression can be described by the Nishi–Wang[16] equation:

$$1/T_m = 1/T_m{}^\circ - (R/\Delta H_u)(V_2/V_1)\chi_{12}(\phi_1)^2 \qquad (1)$$

where subscripts 1 and 2 refer to the amorphous and crystalline components, respectively, T_m and $T_m{}^\circ$ are the melting temperatures of the blend and pure crystallizable polymer, respectively, V is the repeating unit molar volume, ΔH_u is the repeating unit melting enthalpy, ϕ is the volume fraction, and χ_{12} is the Flory–Huggins (polymer–polymer) interaction parameter. In miscible blends, a plot of $1/T_m$ as a function of $(\phi_1)^2$ is expected to be linear. Equation 1 is usually applied to the experimental data using weight (w) instead of volume fractions (ϕ), on the assumption that the blend components have similar amorphous density. In PHBV/a-PHB blends, this assumption is obviously correct.

The T_m results of PHBV/a-PHB blends were plotted according to eq 1 (Figure 2). The linear fit was satisfactory for the blend compositions studied, and the interaction parameter χ_{12} could be calculated from the slope of the linear regression through the experimental data, after independent evaluation of ΔH_u. This latter value was obtained by combining melting enthalpy measurements with crystallinity degree determinations on pure PHBV: the ratio of the experimental melting enthalpy (ΔH_m) to the crystallinity degree (X_c by WAXS, Table 1) yielded the melting enthalpy of 100% crystalline PHBV ($\Delta H_m{}^\circ = 109$ J/g), and hence ΔH_u. The $\Delta H_m{}^\circ$ value obtained for the PHBV used in this work (10 mol % of HV units) was consistent with previous results on PHBV copolymers with different HV contents.[17] From the slope of the plot in Figure 2, a negative polymer–polymer interaction parameter was obtained ($\chi_{12} = -0.32$), indicating that miscibility of PHBV with a-PHB was promoted by effective polymer–polymer interactions.

The degree of crystallinity of PHBV/a-PHB blends, estimated from both WAXS analysis and DSC measurements (ratio of blend melting enthalpy to $\Delta H_m{}^\circ$ of PHBV), is reported in Table 1 and decreases with increasing content of amorphous a-PHB component. The presence of the atactic component in the blends may either simply “dilute” the crystalline phase or to some extent also inhibit PHBV crystallization. In order to answer this question, the melting enthalpy per gram of PHBV present in each blend [ΔH_m(PHBV)] was calculated (Table 1). The values obtained were practically constant, showing that, although the overall crystallinity degree of the blends changed in the order PHBV > 90/10 > 80/20 > 50/50 (PHBV/a-PHB), the ability for crystallization of the bacterial polymer remained unaltered (i.e. PHBV crystallized to the same extent as in the pure state, independent of blend composition).

FTIR spectroscopy confirmed the decrease of crystallinity of PHBV/a-PHB blends with increasing a-PHB content. Blends with varying composition showed a gradual change in the relative intensities of a pronounced shoulder (at 1760–1740 cm^{-1}) and of a rather sharp band (at 1724 cm^{-1}), previously attributed to amorphous and preferred crystalline conformations, respectively.[18]

The rate of isothermal crystallization from the melt of PHBV/a-PHB blends was studied by hot-stage optical microscopy. Both the natural copolymer PHBV and PHBV/a-PHB blends crystallized with spherulitic morphology. At all compositions (0–50% a-PHB) and crystallization temperatures explored ($50 < T_c < 130$ °C), space-filling spherulites were observed to grow at a constant rate. This result implied[19] that the composition of the melt remained constant during the crystallization process and that the amorphous component did not migrate to enrich the melt but remained trapped within the spherulite in the proximity of growing lamellae (either interlamellarly or between bundles of lamellae). The spherulitic radial growth rate (G) of blends and plain PHBV is reported in Figure 3 as a function of crystallization temperature. The overall crystallization curve is depressed by increasing amounts of a-PHB, as expected if a polymer crystallizes from a homogeneous blend. The crystallization rate depression becomes less evident at high T_c, practically vanishing above 110 °C, and the temperature of maximum crystallization rate shifts to higher values with increasing a-PHB content. A similar behavior was reported in an early work by Keith and Padden for blends of isotactic and atactic polystyrene[20] and more recently for blends of bacterial and synthetic PHB.[11,13]

The foregoing experimental results show that blends of bacterial PHBV and synthetic a-PHB are miscible in

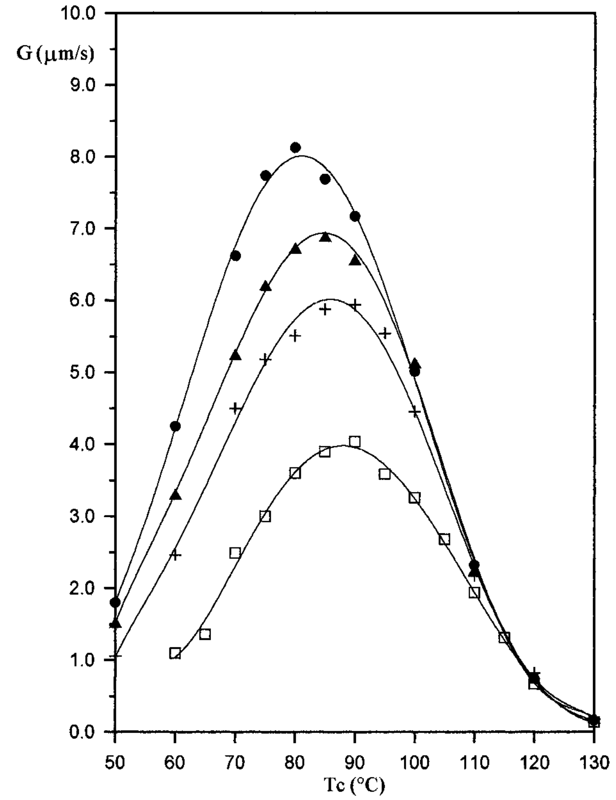

Figure 3. Spherulitic radial growth rate (G) as a function of crystallization temperature for PHBV (●) and PHBV/a-PHB blends: 90/10 (▲); 80/20 (+); 50/50 (□).

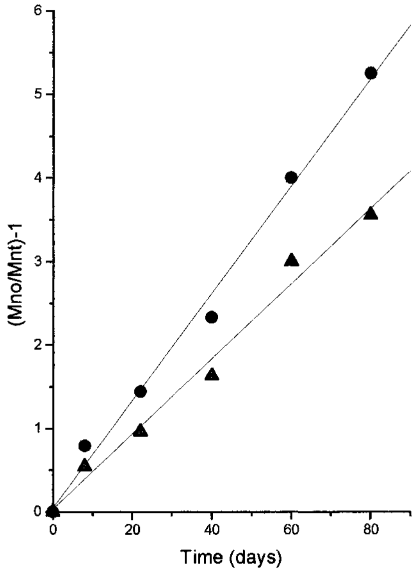

Figure 4. Average number of bond cleavages per polymer molecule (N) as a function of time of hydrolytic degradation: (●) pure a-PHB; (▲) pure PHBV.

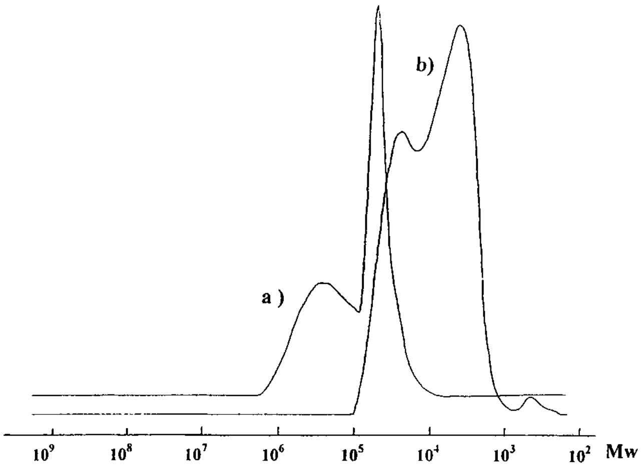

Figure 5. GPC traces of PHBV/a-PHB (50/50) blend: (a) before hydrolytic degradation; (b) after 80 days of exposure to phosphate buffer.

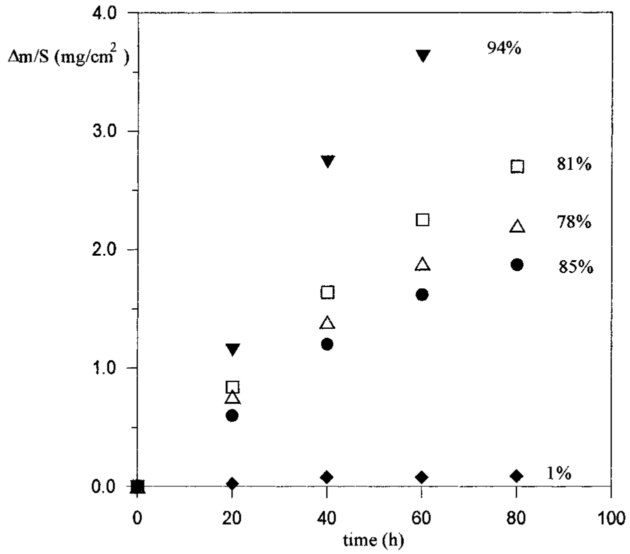

Figure 6. Normalized weight loss as a function of time of the exposure to the enzymatic solution of PHBV (●), a-PHB (◆), and PHBV/a-PHB 90/10 (△), 80/20 (□), and 50/50 (▼). (Total weight loss after biodegradation is reported in the figure.)

the melt in the range of compositions investigated (10–50% a-PHB). These results are similar to those previously reported on the miscibility of bacterial PHB with synthetic analogues of PHB[11,13] and agree with earlier data on blends of bacterial PHBV with bacterial PHB[21,22] which showed that the two biopolymers were miscible, provided that the 3HV content in PHBV was low (in the present PHBV/a-PHB blends, the 3HV content in PHBV was 10 mol %).

The tensile mechanical properties of PHBV/a-PHB blends were investigated (Table 1). The elastic modulus gradually decreased with increasing a-PHB content, concomitant with the decrease of blend crystallinity. The changes of tensile strength and elongation at break became substantial in blend 50/50, where the elongation at break increased 30-fold with respect to that of pure PHBV. This result indicates that the tensile properties of the brittle biopolymer may be remarkably improved through blending with synthetic amorphous PHB.

Hydrolytic Degradation. The hydrolytic degradation of the investigated polymers and their blends was carried out in phosphate buffer at 70 °C and pH 7.4. A systematic decrease of molecular weight of pure PHBV and a-PHB was observed during the degradation in phosphate buffer, and the average number of bond cleavages per polymer molecule (N) was calculated according to Doi et al.:[23]

$$N = [M_n(0)/M_n(t)] - 1 = k_d P_n(0)t \qquad (2)$$

where $M_n(0)$ and $M_n(t)$ are the number-average molecular weights of polyesters at time 0 and time t, k_d is the rate constant of hydrolytic degradation, and $P_n(0)$ is the number-average degree of polymerization at time zero.

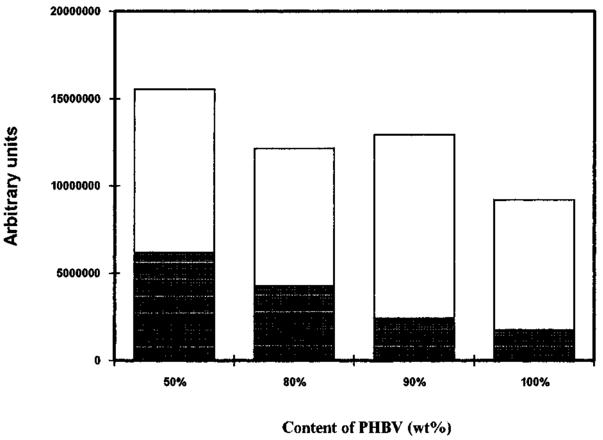

Figure 7. Distribution of 3-hydroxybutyric acid and the dimer of 3-hydroxybutyric acid liberated after 12 h of enzymatic degradation of PHBV and PHBV/a-PHB blend films in Tris-HCl solution (pH = 8) containing PHB-depolymerase A from *Pseudomonas lemoignei* at 37 °C: (■) 3HB; (□) 3HB−3HB.

A linear dependence of N on degradation time was observed for both polymers studied (Figure 4), which indicates that the decrease of molecular weight is caused by random scission of polyester chains.[23] The hydrolytic degradation rate constant k_d estimated for PHBV was equal to 4.1×10^{-5} day^{-1} while that of a-PHB was equal to 14.7×10^{-5} day^{-1}. Synthetic a-PHB degrades faster than PHBV; however, the differences in the degradation rates are not as pronounced as in the case of degradation of synthetic a-PHB compared with that of natural PHB.[7] The degradation rate differences can be due to the different degrees of crystallinity of these polymers. Synthetic a-PHB, which is completely amorphous, shows the fastest rate of hydrolytic degradation; bacterial PHB, which has the highest X_c, is the slowest degrading polymer, whereas PHBV having X_c lower than bacterial PHB[17] shows an intermediate degradation rate.

The GPC traces of PHBV/a-PHB blend (50/50) film before and after 80 days of hydrolytic degradation are shown in Figure 5. Due to the overlapping of the GPC signals of individual blend components during the hydrolytic degradation process, it was difficult to quantify the polymer M_n changes. Nevertheless, pronounced shifts to lower molecular weight values are shown in Figure 5 for both PHBV and a-PHB components.

Enzymatic Degradation. The blends containing 10, 20, and 50% a-PHB as well as PHBV and a-PHB films were subjected to enzymatic biodegradation using PHB-depolymerase A from *P. lemoignei*. The results are shown in Figure 6 as normalized weight loss ($\Delta m/S$) vs time of exposure. The enzyme used in this work biodegraded the bacterial PHBV, as expected,[24] whereas no appreciable weight loss was observed for synthetic atactic PHB. After each exposure time, the blends showed a higher weight loss than the pure biopolymer (PHBV). The weight loss was greater the higher the a-PHB content.

It is known that the biodegradation rate of bacterial PHB strongly depends on crystallinity degree[25] and morphology.[15,26] Consequently, the increase of biodegradation rate observed in the blends with increasing a-PHB content (Figure 6) could be tentatively attributed to the parallel steady decrease of the overall crystallinity. However, the ΔH_m(PHBV) values in Table 1 demonstrated that the bacterial polymer crystallized in the blends to the same extent as in the pure state, showing that a change of PHBV crystallinity could not be invoked to explain the observed increase of biodegradation rate. Moreover, weight loss values at the end of the biodegradation experiments were extremely high (viz. 94% in blend 50/50, Figure 6), clearly indicating that, in addition to degradation of the bacterial component, hydrolysis and solubilization of atactic PHB also occurred in PHBV/a-PHB blends.

The HPLC analysis of acidified solutions of the degradation products of PHBV/a-PHB blends revealed the presence of 3-hydroxybutyric acid (3HB) and its dimer (3HB−3HB). The distribution of monomer and dimer liberated after 12 h of enzymatic degradation of blends and PHBV films is shown in Figure 7. The 3-hydroxyvaleric acid (3HV) and the corresponding

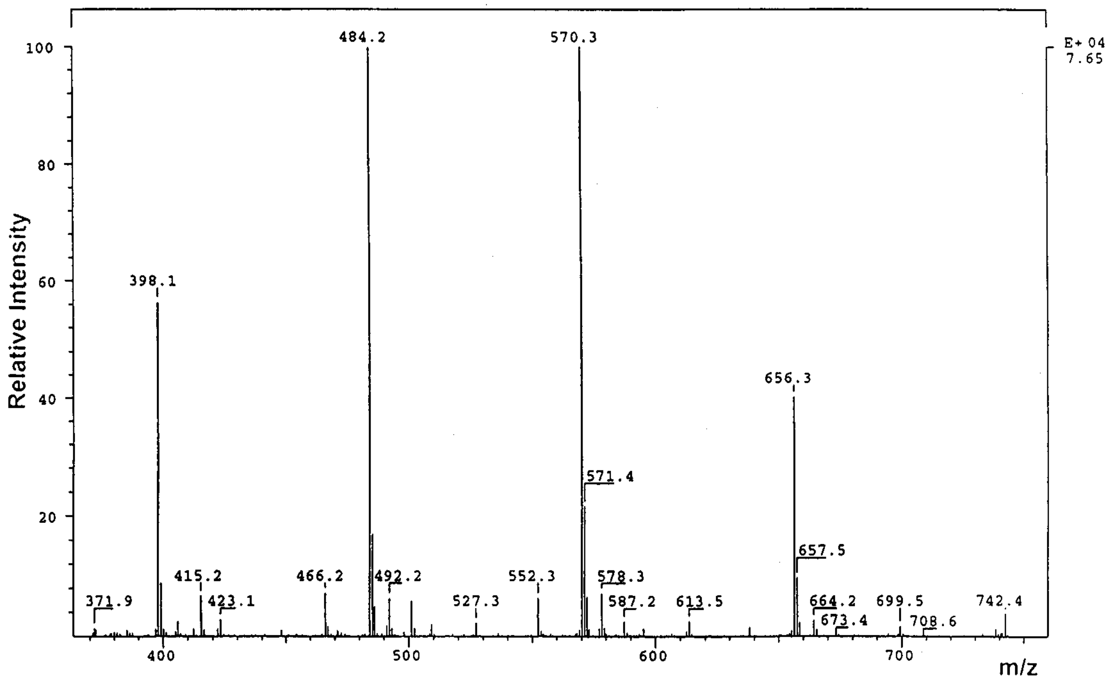

Figure 8. APCI-MS spectrum of PHB oligomers (higher than dimer) formed after 12 h of enzymatic degradation of PHBV/a-PHB (50/50) blend.

dimers 3HV−3HV, 3HV−3HB, and 3HB−3HV were not detected by HPLC analysis of water-soluble degradation products of PHBV/a-PHB blends. This is most probably due to the fact that the PHBV used for the preparation of the blends contained only 10% of HV units. Moreover, Abe et al.[27] reported that the rate of enzymatic hydrolysis of the ester bond of the 3HV−3HV sequence was much slower than the rate of ester bond hydrolysis of the 3HB−3HB sequence. Therefore, the products of 3HB−3HB bond scission are expected to be prevailing and the share of those formed via 3HV−3HV and 3HB−3HV bond scission to be smaller.

Higher oligomers of PHB were detected using APCI-MS analysis of the solutions collected during degradation of PHBV/a-PHB blends. Figure 8 presents the positive ion APCI-MS spectrum of oligomers higher than dimer in the solution analyzed after 12 h of enzymatic degradation of the PHBV/a-PHB 50/50 blend. It shows intense signals of protonated molecular ions in the range of $m/z = 398.1$ up to 742.4. The main peaks in the spectrum show a peak-to-peak mass increment of 86 g mol^{-1}, which is equal to the molecular weight of the PHB repeating unit. The first peak of this mass series (Figure 8) may be attributed to the Tris salt of the PHB trimer, $HO[CH(CH_3)CH_2C(O)O]_2CH(CH_3)CH_2C(O)$-$ONC_4H_{11}O$ of $M_n = 397.42$, which appears as a protonated molecular ion at $m/z = 398.1$. The following peaks may be assigned to this salt of PHB higher oligomers up to heptamer (Figure 8). The APCI-MS analysis of solutions collected after various enzymatic degradation times of PHBV/a-PHB blends revealed the presence of the corresponding oligomers with the size up to heptamer. In contrast, APCI-MS analysis of the solution collected after enzymatic degradation of natural PHBV demonstrated the absence of oligomers higher than dimer. Moreover, no low molecular weight oligomers were observed by APCI-MS analysis of the solution collected after 100 h of incubation of a control a-PHB sample in Tris-HCl buffer without enzyme at 37 °C.

The APCI-MS results were confirmed by electrospray ionization mass spectrometry (ESI-MS). The negative ESI-MS spectra of the solutions collected after enzymatic degradation of PHBV/a-PHB blends revealed the presence of peaks of hydroxybutyric acid anion at $m/z = 103$, its dimer at $m/z = 189$, trimer at $m/z = 275$, tetramer at $m/z = 361$, pentamer at $m/z = 447$, and hexamer at $m/z = 533$. The small peak at $m/z = 117$, attributed to hydroxyvaleric acid anion, was also observed. Again, no oligomers higher than dimer were detected by ESI-MS analysis of the solution collected after enzymatic degradation of natural PHBV.

NMR analysis of a-PHB provided strong evidence that in the synthetic polymer used in this work the (R) and (S) units were distributed in a random fashion along the chain. It was reasonable to assume that attack to such a macromolecule by PHB-depolymerases could occur only at the ester linkages between (R) repeating units. The probability (p) of formation of oligomers higher than dimer was calculated on the assumption that the distribution of (R) and (S) units along the a-PHB polymer chain followed Bernoullian statistics and that all ester linkages between (R)−(R) units of a-PHB were hydrolyzed by the enzyme. It was found that p decreased from 31.25×10^{-3} for trimer to 7.81×10^{-3} for heptamer. This result shows that the probability of formation of the heptamer—an oligomer that was indeed revealed as one of the biodegradation products of the present blends—is only slightly lower (1/4) than that of the trimer.

The results of this work show that PHB-depolymerase A from *P. lemoignei* behaves as an *endo* enzyme toward the a-PHB component during degradation of PHBV/a-PHB blends, producing oligomers higher than the dimer. PHB oligomers (up to tetramer) were previously detected by HPLC analysis as products of enzymatic degradation of synthetic PHB with different tacticities[28] and of their blends with bacterial PHB,[14] carried out in the presence of PHB-depolymerase from *P. pickettii*. Recently, Timmins et al.[29] revealed 3-hydroxybutyric acid, its dimer, and its trimer as the water-soluble biodegradation products of synthetic PHB with different tacticities, formed using PHB-depolymerases from *P. lemoignei* and *Aspergillus fumigatus*. To our knowledge, the present work is the first one reporting the identification of water-soluble oligomers up to heptamer as products of enzymatic biodegradation of a-PHB blends with a bacterial PHA. The mass spectrometric techniques used in this work may be considered as a powerful tool in the investigation of the mechanism of enzymatic hydrolysis of PHAs through identification of the degradation products.

Another interesting observation was that a-PHB biodegraded in a blend with bacterial PHBV, whereas its degradation was totally prevented when this synthetic polymer was exposed alone to the enzymatic attack (Figure 6). A very similar biodegradation behavior was reported recently for blends composed of bacterial PHB and synthetic a-PHB in the presence of PHB-depolymerase from *P. pickettii* by Abe at al.[14] The stability toward enzymatic degradation of synthetic a-PHB was explained by the absence of crystalline domains in this polymer able to provide stable binding sites for the enzyme molecules.[14] The biodegradability of bacterial PHB/a-PHB blends was attributed to the presence of a crystalline phase provided by the bacterial component.

Our results presented now confirm the observations of Abe et al.[14] In spite of the fact that different kind of enzyme (PHB-depolymerase from *P. lemoignei* vs *P. pickettii*) were used, the same biodegradation behavior was displayed by blends, where not only the enzyme used but also the bacterial PHA (PHBV vs PHB) was changed. Therefore, one may conclude that the presence of a partially crystalline PHA in a blend is essential for enzymatic degradation. The assumption that the presence of a crystalline phase is a general requirement for the enzymatic hydrolysis of blends or copolymers of synthetic a-PHB is supported by the results of recent investigations of amorphous/crystalline block copolymers of β-butyrolactone, to be published soon.

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