# Crystallinity-Induced Biodegradation of Novel [(*R,S*)-*β*-Butyrolactone]-*b*-pivalolactone Copolymers

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ABSTRACT: Novel model block copolymers of (*R,S*)-*β*-butyrolactone with pivalolactone (PVL) are prepared in order to define the effect of crystalline domains provided by poly(pivalolactone) on the biodegradability of atactic poly(*β*-butyrolactone), a-PHB. The "living" a-PHB is synthesized from racemic *β*-butyrolactone, in the presence of potassium alkoxide/18-crown-6 complex, and such a living polymer is applied for polymerization of PVL, yielding block copolymers, a-PHB-*b*-PPVL, of tailored molecular weight and composition. The copolymers contain an amorphous phase with $T_g = 5$ °C, associated with the a-PHB block, and a high melting crystalline phase, whose amount increases with PPVL content. Films of copolymers containing 9 (PVL9), 17 (PVL17), and 23 mol % of PPVL (PVL23) are exposed to PHB-depolymerase A from *Pseudomonas lemoignei* (37 °C, Tris−HCl buffer pH = 8). While plain a-PHB does not biodegrade, the biodegradation rate of a-PHB-*b*-PPVL copolymers increases (PVL9 ≪ PVL17 < PVL23) along with the increase of crystalline PPVL domains. The biodegradation rate of PVL23 is similar to that of natural (crystalline) PHB. On the basis of a comparison of a-PHB-*b*-PPVL composition changes (by $^1$H NMR) with weight loss during biodegradation experiments, it is concluded that in the copolymers studied only the a-PHB block is attacked by the enzyme and that the crystalline block of nonbiodegradable PPVL efficiently promotes enzymatic attack to a-PHB, by providing a binding support to the enzyme.

## Introduction

The anionic polymerization of *β*-butyrolactone (BL) is the powerful synthetic method to obtain poly(3-hydroxybutyrate) and its block and graft copolymers.[1−3] Earlier results[4] had shown that synthetic high molecular weight (atactic) poly((*R,S*)-3-hydroxybutyrate), a-PHB, prepared using supramolecular complexes of alkali metal alkoxides,[5] underwent enzymatic attack when blended with a natural (isotactic) crystalline poly(3-hydroxyalkanoate), PHA, but did not biodegrade alone. Abe et al.[6] proposed that in order to hydrolyze the chains of the amorphous polymer, enzymes needed stable binding sites, which were provided in the blends by the crystalline domains of the natural PHA. It seemed, therefore, interesting to combine amorphous segments of a-PHB with those of a crystalline synthetic PHA where a-PHB and the PHA, instead of being physically blended, constitute the structural segments of a di-block copolymer.

The novel poly((*R,S*)-*β*-butyrolactone-*b*-pivalolactone) copolymers, a-PHB-*b*-PPVL, have been prepared in order to verify the above hypothesis regarding enzymatic degradation of atactic PHB[6] and namely the ability of the crystalline phase of PPVL to provide a support to the depolymerizing enzymes. In contrast to the PHAs previously blended with a-PHB,[4,6] PPVL should not be attacked by PHB-depolymerases owing to the chemical structure of its repeating unit. This consideration offers the possibility to check if, in the studied copolymers, the biodegradability of the crystalline polyester block is a necessary condition to promote biodegradation of the a-PHB block.

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Pivalolactone (α,α-dimethyl-*β*-propiolactone) (PVL) is a monomer of industrial applications used for the synthesis of a high molecular weight polyester, poly(pivalolactone) (PPVL), characterized by a high degree of crystallinity and a relatively high melting point.[7,8] Several attempts have been taken previously in order to synthesize PPVL block and graft copolymers.[9−11] The recent mechanistic studies on anionic polymerization of *β*-lactones provided the basis for the synthetic approach to novel block copolymers of racemic *β*-butyrolactone with pivalolactone presented herein.[12−15]

In the present work, the "living" poly(*β*-butyrolactone) synthesized in the presence of potassium alkoxide/18-crown-6 complex and having the carboxylate active centers was applied as an initiator responsible for formation of poly(pivalolactone) block. The obtained a-PHB-*b*-PPVL copolymers, with different block lengths, were characterized and subjected to biodegradation studies in the presence of PHB-depolymerase A from *Pseudomonas lemoignei.*

## Experimental Part

**Materials.** *β*-Butyrolactone (BL) (Aldrich) and pivalolactone (3,3-dimethyl-2-oxetanone) (PVL) were dried over calcium hydride for 48 h at room temperature and next distilled under reduced pressure. BL was additionally distilled over Na/K alloy under reduced pressure just before use. The BL and PVL fractions boiling at 47 °C (5 mmHg) and at 48−49 °C (9 mm Hg), respectively, were collected. Potassium methoxide was obtained as described in ref 16. THF was purified as described in ref 17. 18-Crown-6 (Fluka) was purified as described earlier.[1]

**General Procedure of Block Copolymerization.** Into the reactor containing a vigorously stirred suspension of potassium methoxide in THF was added an equimolar amount of 18-crown-6. After 1 h, the BL was added and the reaction mixture was stirred at room temperature till the BL conversion

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was 100% (60−94 h, conversion was determined by $^1$H NMR in $CDCl_3$ solution). Next, the THF solution of the required amount of PVL was added into the "living" BL prepolymer. The PVL concentration was changed in the range 0.1−0.6 mol/L. After a specified period of time (2−24 h), the block copolymerization was terminated by addition of an ethyl ether solution of HCl. Then the obtained product was precipitated in hexane or methanol, recovered by filtration, and dried.

The PPVL reference polymer was prepared via anionic polymerization of PVL, as described in ref 13.

**Film Preparation.** Films for enzymatic degradation were obtained by casting solutions of the polymer in $CH_2Cl_2$ on Teflon plates. Teflon was selected instead of glass owing to the ease of peeling off the film. No solution-cast films were obtained from PPVL due to the very high crystallinity and consequent brittleness of this polyester.[7,8] The same happened with the copolymer with the highest PVL content (48%). Copolymers PVL9, PVL17, and PVL23, yielded acceptable films.

**Experimental Techniques.** $^1$H and $^{13}$C NMR spectra were recorded by using a Varian VXR-300 spectrometer in $CDCl_3$ + TFA (trifluoroacetic acid) with TMS as the internal standard. $^{13}$C NMR spectra of block copolymers were recorded on 10% solutions at 35 °C. In order to obtain a satisfactory signal-to-noise ratio, 30 000 scans were accumulated.

Gel permeation chromatography was performed at 30 °C, using a Spectra Physics 8800 gel-permeation chromatograph with two PL-gel packed columns ($10^3$ Å and 500 Å). THF was used as the eluent at a flow rate of 1 mL/min. Polystyrene standards with low polydispersity (PL-Laboratories) were used to generate a calibration curve. The number average molecular weight $M_n$ of the a-PHB block was determined by GPC and confirmed by NMR; the $M_n$ of the poly(pivalolactone) block was estimated by $^1$H NMR spectroscopy from the intensity ratio of the a-PHB methine group signal at $\delta = 5.26$ ppm and the signal of the PPVL methylene group at $\delta = 4.10$ ppm.

Thermogravimetric (TGA) measurements were made with a Perkin-Elmer TGA7 thermogravimetric analyzer, under nitrogen flow (flow rate = 20 mL/min) with a scan rate of 10 deg/min.

Differential scanning calorimetry (DSC) was performed with a DuPont 9900 thermal analyzer in the temperature range −80 to +225 °C. The temperature scale was calibrated with high-purity standards. The melting temperature ($T_m$) was taken as the peak temperature of the melting endotherm in the first DSC scan at a heating rate of 10 °C/min, while the glass transition temperature ($T_g$) was taken as the midpoint of the stepwise increase of the specific heat associated with the transition, after melt quenching, at a heating rate of 20 °C/min.

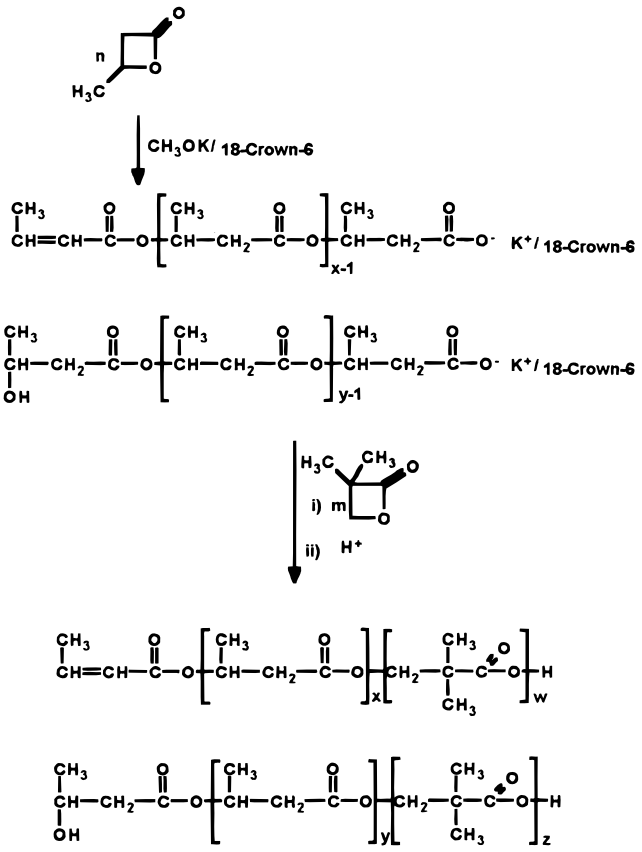
Wide angle X-ray diffraction (WAXS) data were collected using nickel-filtered Cu Kα radiation ($\lambda = 0.1542$ nm, 40 kV, 30 mA), with a Philips PW1050/81 powder diffractometer controlled by a PW1710 unit.

**Enzymatic Degradation.** Film samples (20 × 7 mm$^2$, thickness 8−15 × $10^{-2}$ mm, initial weight 15−25 mg) were incubated at 37 ± 0.1 °C in vials containing 1.5 mL of 50 mM Tris−HCl, pH 8.0, 1 mM $CaCl_2$, in the presence of *P. lemoignei* PHB-depolymerase A (4.2 μg/mL), isolated and purified as described previously.[18] After 8 h, films were removed from the solution, washed with distilled water, and dried under vacuum at room temperature to constant weight (Sartorius RC210D electronic balance; reproducibility ±0.02 mg). This procedure was repeated using a fresh enzymatic solution for each incubation interval. The extent of biodegradation was quantified as weight loss divided by initial sample surface area ($\Delta m/S$). Control tests, carried out in buffer without enzyme addition, showed no appreciable weight losses over the time scale of the biodegradation experiments.

## Results and Discussion

**Synthesis of a-PHB-*b*-PPVL Copolymers.** It was demonstrated previously that high molecular weight (up to $M_n = 40\,000$) atactic poly((*R*,*S*)-hydroxybutyrate) can be prepared using supramolecular complexes of alkali metal alkoxides.[5] It has also been shown that the mechanism of anionic ring-opening polymerization of β-lactones is strongly dependent on the monomer structure and namely on the position of alkyl substituents in the β-lactone ring.[12] Anionic polymerization of PVL proceeds via different mechanisms depending on the initiator employed. Polymerization of PVL initiated with alkali metal alkoxides proceeds *via* alkoxide active species with formation of cyclic oligomers as side products.[12,13] When carboxylates of alkali metals are used as initiators, the polymerization of PVL takes place exclusively on carboxylate active centers.[13] On the other hand, in BL polymerization initiated with alkali metal alkoxides, due to the addition−elimination reaction, taking place at the preinitiation stage, the alkoxide is not incorporated into the polymer chain, and polyesters containing unsaturated and/or hydroxy dead end groups are produced. Polymerization takes place exclusively on carboxylate active centers formed *via* the alkyl−oxygen bond cleavage of β-butyrolactone monomer.[14,15]

**Scheme 1**




In the present study the "living" a-PHB was synthesized from racemic β-butyrolactone in the presence of potassium methoxide/18-crown-6 complex, and was characterized by NMR and GPC. The obtained a-PHB containing either hydroxybutanoic or crotonate dead end groups as well as carboxylate active centers was applied for polymerization of pivalolactone. The reaction pathway to a-PHB-*b*-PPVL copolymers is presented in Scheme 1.

Results of subsequent block polymerization of PVL with a-PHB macroinitiator, prepared in the presence of potassium alkoxide/18-crown-6 complex, are summarized in Table 1.

The results presented in Table 1 indicate that high molecular weight block copolymers of BL with PVL can

**Table 1. Block Copolymers Obtained by Anionic Polymerization of Pivalolactone (PVL) Initiated with Poly(β-butyrolactone) Prepolymer (a-PHB) in THF at Room Temperature**

| sample | $M_n$ of a-PHB block | molar ratio of monomers BL:PVL | yield (%) | block copolymer composition[a] a-PHB:PPVL | $M_n$ of PVL block | $M_n$ of block copolymer[b] |
|---|---|---|---|---|---|---|
| PVL9 | 17 000 | 90:10 | 96 | 91:9 | 1 900 | 18 900 |
| PVL17 | 12 000 | 82:18 | 93 | 83:17 | 2 800 | 14 800 |
| PVL23 | 20 500 | 70:30 | 88 | 77:23 | 7 100 | 27 600 |
| PVL48 | 13 000 | 45:55 | 85 | 52:48 | 13 900 | 26 900 |

[a] Estimated from $^1$H NMR spectra based on the integrals of proton signals corresponding to a-PHB and PPVL units. [b] Obtained from addition of $M_n$ of the two blocks.

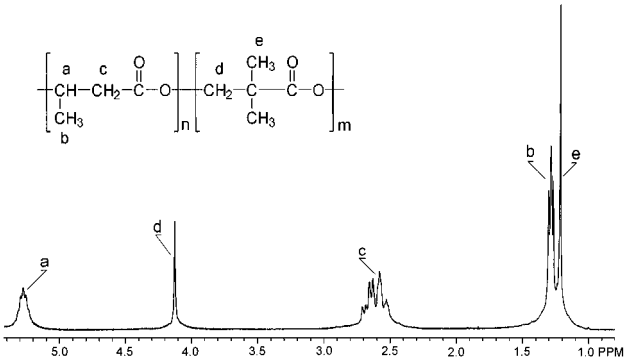



**Figure 1.** $^1$H NMR spectrum of poly(β-butyrolactone)-*block*-poly(pivalolactone) obtained *via* polymerization of PVL initiated by the a-PHB macromonomer with the potassium counterion complexed by 18-crown-6 (sample PVL23; $M_n$ = 27 600; estimated from $^1$H NMR).

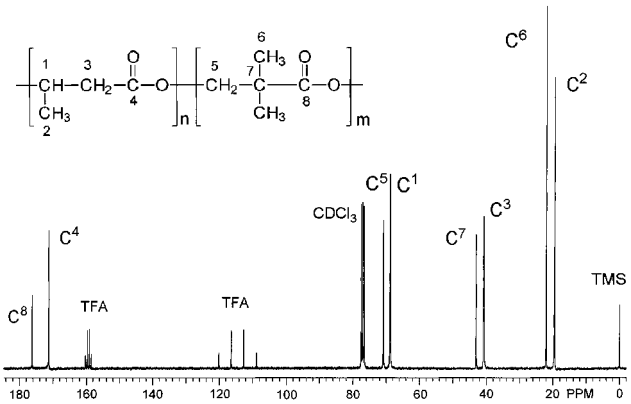



**Figure 2.** $^{13}$C NMR spectrum of poly(β-butyrolactone)-*block*-poly(pivalolactone) (sample PVL23).

be obtained. The yield of block copolymerization depends on the molar ratio of PVL in the reaction mixture and is almost quantitative at relatively low PVL ratio. When the PVL molar ratio in the reaction mixture increases, the yield decreases due to poor solubility in THF of the higher molecular weight PVL block formed.

The $^1$H NMR spectra of block copolymers obtained show signals characteristic of the BL units ($\delta$ = 1.28 ppm $CH_3$, 2.58 ppm $CH_2$, and 5.26 ppm CH) and PVL units ($\delta$ = 1.20 ppm $CH_3$ and 4.10 ppm $CH_2$), respectively (Figure 1).

The $^{13}$C NMR spectra of copolymers obtained reveal the presence of the corresponding signals attributed to the respective carbon atoms of a-PHB and PPVL blocks (Figure 2).

It is also worth noticing that the intensity ratio of signals corresponding to a-PHB block methylene carbon (triads: S = 25%, $H_s$ = 25%, I = 24%, and $H_i$ = 26%) indicates the atactic structure of this block.[19,20] Moreover, the $^{13}$C NMR spectra of diblock copolymers reveal the absence of signals corresponding to an alternating copolymer structure in the carbonyl carbons region,

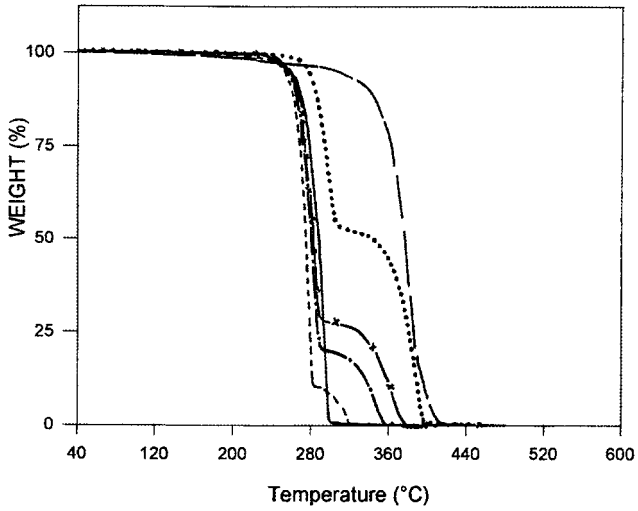



**Figure 3.** Thermogravimetric curves of a-PHB (—, $M_n$ = 16 000), PPVL (— —), and of block copolymers: PVL9 (- - -), PVL17 (— · —), PVL23 (— × —), PVL48 (···).

indicating that transesterification reactions do not take place in this block copolymerization.

Due to very poor solubility of PVL polymer in common solvents, the molecular weight and molecular weight distribution of resulting copolymers could not be determined by GPC. However, the block structure of the obtained copolymers has been confirmed by selective extraction experiments.[21]

The above presented results indicate that BL−PVL diblock copolymers, with various compositions, can be prepared in the way of subsequent anionic copolymerization taking place on carboxylate propagation centers, with the complexed potassium counterion.

**Physical Properties of a-PHB-*b*-PPVL Copolymers.** Thermal properties of both homopolymers (a-PHB and PPVL) and block copolymers with different compositions were investigated by TGA and DSC.

Thermal degradation of a-PHB and PPVL occurred in a single weight loss step (Figure 3). The temperature of maximum weight loss rate was quite different: 290 °C and 380 °C for a-PHB and PPVL, respectively. These values were in good agreement with previous literature results.[22,23] All copolymers showed two distinct, well-resolved weight loss steps (Figure 3) respectively assigned to thermal degradation of the a-PHB and PPVL block. The magnitude of each weight loss was closely correlated with the content of the corresponding block in the copolymer, according to the composition determined by $^1$H NMR (Table 1).

Films of copolymers PVL9, PVL17, and PVL23 were characterized by DSC and showed a melting process whose entity ($\Delta H_m$) increased with increasing PVL content (see Table 2).

The crystalline modifications of PPVL have been extensively studied[8,24−30] and three crystalline phases are described in the literature, characterized by differ-

**Table 2. Thermal Properties of a-PHB-*b*-PPVL Block Copolymers**

| sample | $T_m$ (°C) | $\Delta H_m$[a] (J/g) | $\Delta H_m$ (PPVL)[b] (J/g) |
|---|---|---|---|
| PVL9 | 174 | 13 | 130 |
| PVL17 | 178 | 24 | 126 |
| PVL23 | 212 | 32 | 123 |
| PPVL | 212−218[c] | 140 | |

[a] Melting enthalpy per gram of whole sample. [b] Melting enthalpy per gram of PPVL. [c] Two-peak endotherm.

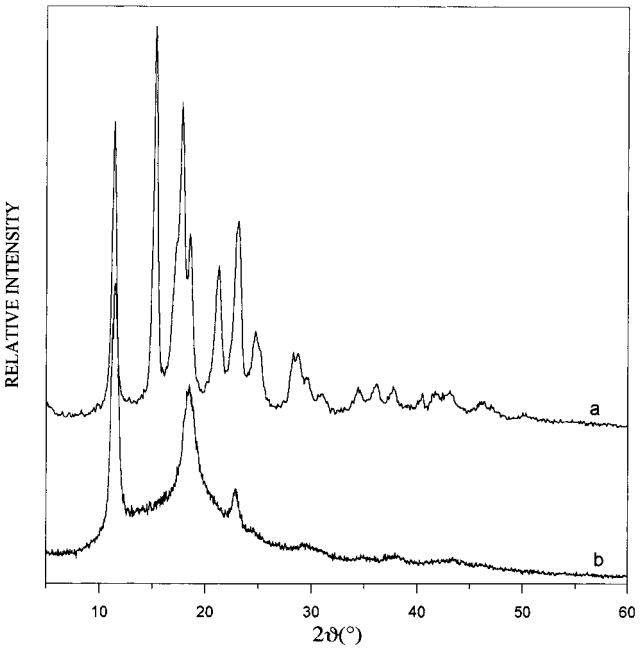



**Figure 4.** X-ray diffraction spectra of PPVL (a) and of block copolymer PVL23 (b).

ent melting temperatures: the α and γ phases contain 2/1 helices with an antiparallel chain packing[28] and the β-phase, induced by stretching from the α-phase, has planar zigzag chain conformation.[27] WAXS measurements of copolymer films (PVL9, PVL17, and PVL23) and of PPVL in powder form, showed rather different diffraction spectra. As an example, Figure 4 compares the WAXS spectra of PPVL and PVL23. While the spectrum of the homopolymer closely agreed with that reported in the literature for the α-phase,[24,26] all copolymer films showed a diffraction spectrum containing three main diffraction peaks at $2\theta$ =11.4°, $2\theta$ = 18.5°, and $2\theta$ = 22.8°, superimposed on a broad amorphous halo, which could not be straightforwardly assigned to any of the mentioned crystalline modifications. In the copolymers the intensity of the reflections decreased in the order PVL23 > PVL17 > PVL9, consistent with the decrease of $\Delta H_m$ reported in Table 2. The melting enthalpy of copolymers was also calculated with respect to the content of PPVL (PVL9 = 10 wt %, PVL17 = 19 wt %, PVL23 = 26 wt %), and the results [$\Delta H_m$(PPVL)] are shown in Table 2. The close values obtained for the three copolymers indicate that in a-PHB-*b*-PPVL PPVL crystallizes to the same extent, independent of block length.

The DSC melting endotherm of PPVL contained two closely located maxima at temperatures lower than those reported by Prud'homme and Marchessault[26] for the various crystal modifications of PPVL. The block copolymer films, on the contrary, showed a single-peak endotherm whose temperature decreased with decreasing content of PPVL. Since the three copolymers showed an analogous WAXS spectrum, the decrease of

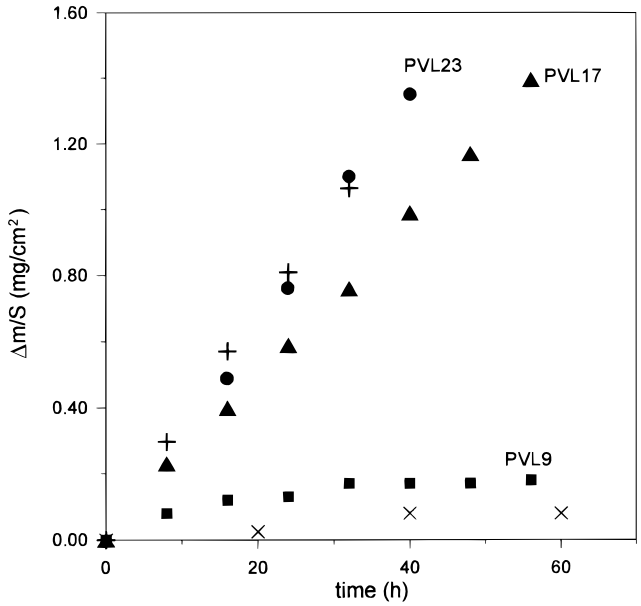



**Figure 5.** Normalized weight loss as a function of exposure time to an enzymatic solution of PVL9 (■), PVL17 (▲), and PVL23 (●). Biodegradation results on atactic PHB (×, $M_n$ = 38 000, see also ref 4) and bacterial PHB (+, $M_n$ = 131 000, $M_w/M_n$ = 4.11, compression-molded film) are shown for comparison.

$T_m$ was attributed to loss of perfection of the crystals with shortening of PPVL block, rather than to changes of crystal phase type.

When the copolymers were quenched below room temperature, on a subsequent DSC scan a well-defined glass transition was observed in the $T_g$ range of plain a-PHB (about 5 °C), whose intensity increased with a-PHB content. No glass transition attributable to the PPVL block was detected, due to the rather low PPVL content of the copolymers and to the well-known difficulty to inhibit crystallization of this polyester by quenching. As a matter of fact, the few conflicting data on the glass transition of PPVL available in the literature were obtained from relaxation techniques rather than by DSC (6[31] and 96 °C[32] from dynamic-mechanical measurements, 137 °C[33] from dielectric measurements).

In conclusion, in a-PHB-*b*-PPVL copolymers, the a-PHB block contributed an amorphous phase with a glass transition below room temperature, whereas the PPVL block provided a crystalline phase whose entity increased with increasing block length.

**Enzymatic Degradation of a-PHB-*b*-PPVL Copolymers.** Films of copolymers PVL9, PVL17, and PVL23 were subjected to enzymatic degradation using PHB-depolymerase A from *P. lemoignei*. The biodegradation results (Figure 5) showed the continuous weight loss of samples PVL17 and PVL23 during the course of the whole experiment. Conversely, the copolymer containing only 9% of PPVL exhibited a slow weight loss at the beginning of the experiment and after about 20 h of exposure its weight remained unchanged. After each retrieval from the enzymatic solution the extent of biodegradation increased in the order PVL9 ≪ PVL17 < PVL23. Based on linear regression of the data of Figure 5, biodegradation rates of $2.4 \times 10^{-2}$ and $3.4 \times 10^{-2}$ mg/(cm$^2$ h) were calculated for PVL17 and PVL23, respectively. These results show that the biodegradation process of a-PHB-*b*-PPVL copolymers accelerates with increasing PVL content. The ratio of the above rate values for PVL23 and PVL17 (1.4) is equal to the ratio of the PPVL content (weight fraction)

**Table 3. Biodegradation Results of a-PHB-*b*-PPVL Block Copolymers**

| sample | total weight loss[a] (%) | % PVL content (calcd)[b] | % PVL content (from $^1$H NMR) |
|---|---|---|---|
| PVL17 | 34 | 29 | 27 |
| PVL23 | 27 | 37 | 34 |

[a] At the end of biodegradation experiment (PVL17, 56 h; PVL23, 40 h). [b] From the weight loss data, assuming biodegradation of the a-PHB block only.

in the two copolymers. The biodegradation rate of PVL23 was comparable to that of bacterial PHB (compression molded film) tested under the same experimental conditions (Figure 5).

The samples PVL17 and PVL23 lost 34% and 27%, respectively, of their initial weights at the end of the biodegradation experiments (Table 3). The PPVL repeating unit of the block copolymers investigated substantially differed from the (*R*)-3-hydroxybutyrate unit of biological PHB, which is the natural substrate for PHB-depolymerase. It is reasonable to expect that the bulkiness of the two methyl groups at the α-carbon of PPVL repeating units should create serious steric problems for enzymatic hydrolysis of the PPVL ester linkages. Hence, on the assumption that the observed weight loss was only due to degradation of the a-PHB block (i.e., no enzymatic attack to PPVL), the expected composition of samples PVL17 and PVL23 after biodegradation was calculated and reported in Table 3. These calculated composition values were compared with the experimental ones determined by $^1$H NMR measurements after biodegradation (Table 3). The excellent agreement between experimental and calculated composition demonstrates that in a-PHB-*b*-PPVL only the a-PHB block underwent enzymatic attack and validates the suggestion that PHB-depolymerase A from *P. lemoignei* is incapable of hydrolyzing the PPVL block.

Earlier experiments revealed that in the presence of PHB-depolymerases from either *P. lemoignei*,[4] *Pseudomonas pickettii*,[6] or *Penicillium funiculosum*[34] amorphous a-PHB does not undergo biodegradation (compare with Figure 5). Hence, neither of the components of the present block copolymers is intrinsically biodegradable. Evidence that in a-PHB-*b*-PPVL copolymers the a-PHB block is hydrolyzed by the enzyme suggests that the nondegradable PPVL block plays an important role in inducing biodegradation of a-PHB. This idea is confirmed by the observed increase of biodegradation rate with PPVL content (Figure 5).

The key to explain why a certain amount of PPVL was needed in the block copolymers to trigger biodegradation of atactic PHB could be the high crystallinity of PPVL. In fact previous work on blends of bacterial PHAs with atactic PHB[4,6] showed that the presence of the crystalline natural PHA was an indispensable condition for enzymatic hydrolysis of the a-PHB segment.

In all known PHB-depolymerases a C-terminal segment of the amino acid sequence, rich in hydrophobic residues, has been identified[35,36] as the "binding domain" to the natural solid substrate [partially crystalline poly((*R*)-3-hydroxybutyrate)]. Abe et al.[6] have attributed the observed nonbiodegradability of a-PHB to the lack of stable binding sites in this amorphous polymer. In the blends with natural PHAs, where a-PHB was found to be biodegradable,[4,6] stable sites for enzyme binding should be provided by the crystalline phase of the natural polymer. However, the fact that the PHA biodegraded concomitantly to a-PHB opened the question whether the role of the natural polyester was that of a mere binding support. The present results on a-PHB-*b*-PPVL demonstrate that, although the crystalline PPVL block does not undergo biodegradation, it efficiently promotes enzymatic attack to a-PHB, as shown by an increase of the biodegradation rate from zero (in plain a-PHB) to a value comparable to that of natural crystalline PHB (in PVL23). Hence the crystalline polymer simply plays an enzyme-supporting function in the a-PHB biodegradation process. Evidence that PHB-depolymerase binds to PPVL was obtained by suspending PPVL powder (no films were available, see Experimental Part) in Tris−HCl buffer, adding PHB-depolymerase (in appropriate amount to allow subsequent activity measurements[18]), and centrifuging after 20 min. In the supernatant a considerable reduction of PHB-depolymerase activity was observed, demonstrating that the enzyme binds to the crystalline polymer.

In order to gain further understanding of this phenomenon, it would be interesting to know if there are any specific structural requirements to be met by the crystalline phase of the "supporting polymer" in order to induce enzyme binding. Substrate specificity of several PHB-depolymerases, including PHB-depolymerase A from *P. lemoignei*, have been previously investigated.[37,38] Mukai et al.[37] found that, in addition to biodegrading the natural biopolymer poly((*R*)-3-hydroxybutyrate), the enzymes were active toward linear polyesters like poly(β-propiolactone) and poly(γ-butyrolactone) but not toward poly(δ-valerolactone) or poly(ϵ-caprolactone). Later work using PHB-depolymerase from *Alcaligenes faecalis*[39] on films of various aliphatic polyesters showed that the enzyme was able to bind to the polyester surface even when it was incapable of hydrolyzing the polymer chain, as in the case of poly(ϵ-caprolactone). It was concluded that the binding domain of PHB-depolymerase from *A. faecalis* was nonspecific, whereas the active site of this enzyme was specific for the hydrolysis of selected PHAs.

The above considerations together with the present results on a-PHB-*b*-PPVL copolymers suggest that in the biodegradation reaction the enzyme binding should not be significantly affected by the specific crystalline phase structure of the polyester surface. However, attention is called to the fact that PPVL belongs to the general class of poly(β-hydroxyester)s, all of which have a crystalline conformation[40] very similar to that of crystalline natural PHB. In order to get a better insight into this phenomenon, further investigations on the biodegradability of atactic PHB in the presence of crystalline polymers with a range of different crystal structures will be undertaken.

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