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ENZYMATIC DEGRADABILITY OF POLY(β-HYDROXYBUTYRATE) AS A FUNCTION OF TACTICITY

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ABSTRACT

The enzymatic degradation of films of synthetic poly[(*R,S*)-β-hydroxybutyrate], PHB, of various tacticities was investigated by weight loss measurement. Extracellular PHB depolymerases of the bacterium *Pseudomonas lemoignei* and the fungus *Aspergillus fumigatus* M2A were used. In both enzyme systems the nearly atactic samples showed the greatest weight loss; this maximum occurred at a slightly lower percent isotacticity in the *P. lemoignei* system than in the *A. fumigatus*. The maximum degradation rate for the *P. lemoignei* system was double that of the *A. fumigatus*; natural PHB degraded an order of magnitude more rapidly in both enzyme systems. As isotacticity increased, synthetic PHB showed decreased degradation while syndiotactic samples did not appreciably degrade. Results are interpreted in terms of both crystallinity and stereochemistry; the *A. fumigatus* system is more affected than the *P. lemoignei* by the presence of the *S* repeat units. Total weight loss data suggest that the enzymes are capable of *endo* cleavage.

INTRODUCTION

Poly(β-hydroxybutyrate), PHB, is a biodegradable polyester made by bacteria as a storage material [1–3]. In the natural polymer, all chiral carbon atoms have the *R* configuration, giving perfectly isotactic polymer. Racemic monomer can be polymerized to yield synthetic PHB with a variety of tacticities: stereoblock isotactic [4–8], atactic [9, 10], and syndiotactic [11–13]. Investigating the enzymatic degradability of these stereocopolymers allows greater understanding of the fundamental principles controlling the biodegradation of PHB.

EXPERIMENTAL

The synthetic PHB used in this study was made by ring-opening polymerization of (*R,S*)-β-butyrolactone using a methylaluminoxane catalyst [11]. It was then fractionated according to tacticity, and solvent-cast into films. Bacterial PHB, obtained from Marlborough Biopolymers (Billingham, UK), was similarly cast. The enzymes used were extracellular PHB depolymerases isolated from the bacterium *Pseudomonas lemoignei* [14] and the fungus *Aspergillus fumigatus* [15]. Degradation was examined by placing the polymer films into buffer containing a fixed activity of enzyme, and monitoring weight loss normalized to initial film surface area [16]. Control experiments included degradation of bacterial PHB and degradation of each material in the absence of enzyme.

RESULTS AND DISCUSSION

The synthetic racemic PHB films varied in tacticity from 34 to 88% isotactic diads, as determined by integration of the two components of the ^{13}C NMR carbonyl peak. Crystallinities of these materials increased to either side of ~50%

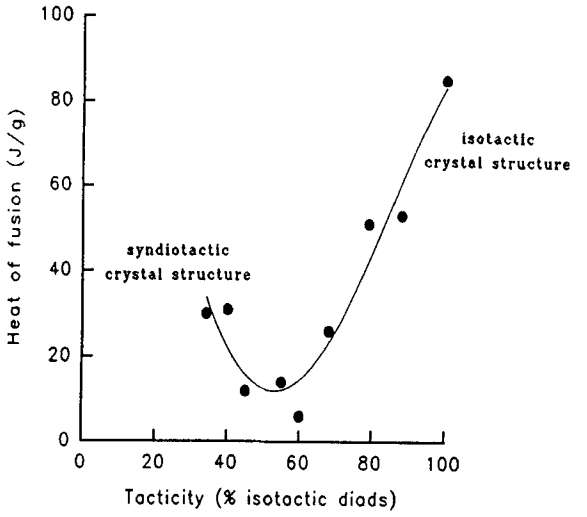

FIG. 1. Crystallinities of PHB films used, as determined by heats of fusion.

isotactic diads as shown in Fig. 1; isotactic and syndiotactic crystal structures were easily distinguished by wide-angle x-ray diffraction [11].

Degradation of Synthetic PHB Films

Figure 2 shows typical results for the bacterial system, plotted as weight loss (mg/cm^2) versus time. Total weight loss for each material is shown as the percentage of initial sample weight. Results for both enzyme systems are summarized in

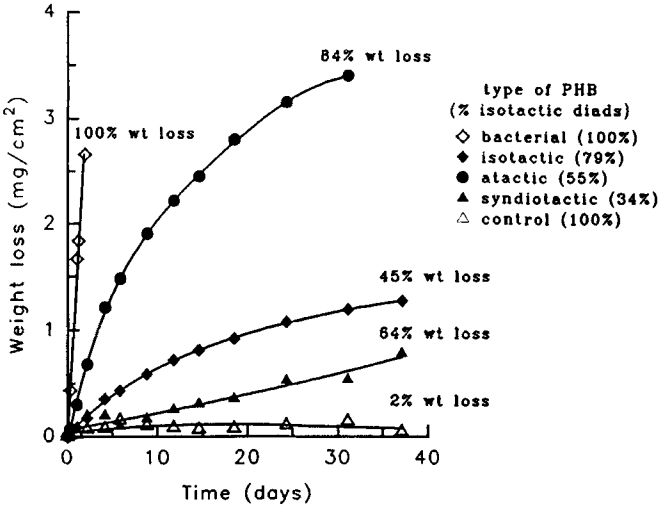

FIG. 2. Weight loss of natural and synthetic PHB by *P. lemoignei* and *A. fumigatus* extracellular depolymerase.

Fig. 3, where values for all replicates of each sample were averaged and plotted as weight loss after 500 hours of degradation time versus isotactic diad content. Control samples, treated identically to the test samples but with no enzyme added, are also shown.

Examination of these results confirms that both crystallinity and regularity of *R* configuration are significant factors in degradation behavior. Previous experiments have shown that decreasing crystallinity increases degradability [17–19], but decreasing the length of the *R* sequence decreases degradability [18, 20, 21]. These two factors act in opposing directions in isotactic racemic PHB, such that an optimal balance is reached at 55–60% isotactic diads; at higher isotacticities, the negative effect of higher crystallinity is greater than the positive effect of higher isotacticity, so degradability decreases. In syndiotactic racemic PHB, crystallinity increases with decreasing isotacticity; as both these factors have a negative effect on degradability, the observed degradation is minimal.

Differences between the two enzyme systems are clearly visible in Fig. 3. The maximum degradation for *A. fumigatus* enzymes is about half that for *P. lemoignei*, despite the fact that both systems degraded bacterial PHB at the same rate. Highly isotactic samples (79–88% isotactic diads) degraded significantly in the *P. lemoignei* enzymes but not in the *A. fumigatus*, and the whole degradation peak occurs at a slightly lower isotacticity for the *P. lemoignei* system. These observations suggest that the *P. lemoignei* enzymes are less hindered by the unnatural *S* unit than are the *A. fumigatus* enzymes.

Total weight loss data can be used to speculate on the enzyme degradation mechanism. Identification of small oligomers as the primary degradation products of bacterial PHB [22–25] suggests that degradation occurs preferentially from the chain ends (*exo* attack). If this were the only mechanism involved, degradation of racemic samples would cease before reaching 50% of the initial sample weight, as

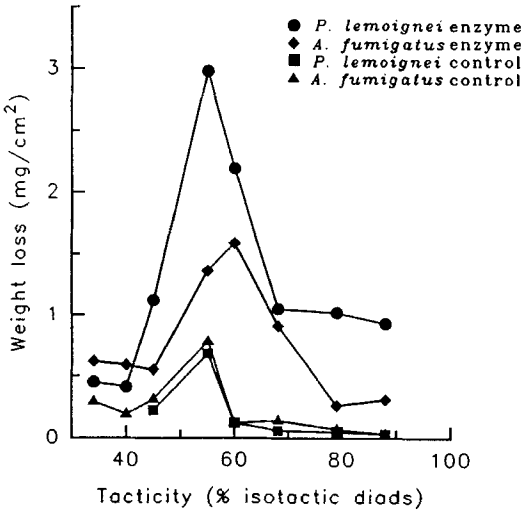

FIG. 3. Weight loss of synthetic PHB after 500 hours of degradation by *P. lemoignei* and *A. fumigatus* extracellular depolymerases.

the enzymes would not be able to penetrate the blocks of *S* units which would accumulate at the chain ends, and these undegradable *S* units comprise 50% of the initial sample mass. However, samples in this study showed total weight losses up to 80% of initial mass, indicating that at least a small amount of *endo* cleavage must occur to provide new, degradable chain ends. That the synthetic racemic samples degrade approximately an order of magnitude less readily than bacterial PHB gives some indication of the relative preference the enzymes have for *exo* cleavage over *endo*.

CONCLUSIONS

This work has shown that depolymerases from both *P. lemoignei* and *A. fumigatus* are capable of degrading synthetic racemic PHB, within the restrictions of crystallinity and stereochemistry. Thus, atactic racemic PHB shows the greatest degradability, highly isotactic racemic PHB degrades less well due to high crystallinity, and syndiotactic racemic PHB shows minimal degradation due to both high crystallinity and low isotacticity. Comparison of the two enzyme systems indicates that the *A. fumigatus* depolymerase is more sensitive than the *P. lemoignei* depolymerase to the stereochemistry of the PHB substrate. Finally, total final weight losses imply that the degradation mechanism includes *endo* attack.

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