Single Crystals and Crystalline Morphology of Synthetic Racemic Poly(β-hydroxybutyrate)

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ABSTRACT: Single crystals of synthetic racemic poly(β-hydroxybutyrate), PHB, of varying tacticities were grown in dilute chloroform/ethanol solutions. The single crystals of highly isotactic synthetic PHB were spear or lath shaped, like those of bacterial PHB; those from essentially atactic polymer were wrinkled but displayed the same electron diffractogram as isotactic PHB. Syndiotactic PHB gave a nondiffracting precipitate of two different morphologies; further examination suggests that these morphologies differ in tacticity. The average overall tacticities of the precipitated and soluble material were determined by ^{13}C NMR and found to be unchanged by the precipitation. This provides evidence that some cocrystallization of the *R* and *S* repeat units can occur and that the single crystals obtained can be correlated with original sample tacticities.

Introduction

Poly(β-hydroxybutyrate), PHB, is a crystalline biopolyester made by a variety of microorganisms as an intracellular carbon and energy reserve[1−3] (Figure 1). Once isolated from the microorganism, the polymer is a thermoplastic material similar in properties to isotactic polypropylene but with the added advantage of being completely biodegradable. This biodegradability gives PHB and other members of the poly(β-hydroxyalkanoate), PHA, family many potential uses in disposable products. The material also has value to synthetic organic chemists as a source of chiral synthons,[4] given that most natural PHA residues have solely an absolute *R* configuration at the point of side-chain attachment.

One of the limitations of PHB for commercial purposes is its high crystallinity, which makes it more brittle than isotactic polypropylene. For most commercial applications, toughness is enhanced by use of copolymers involving varying amounts of β-hydroxyvalerate (HV) residues. While these copolymers maintain high crystallinity due to isodimorphism,[5−7] in poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate), PHBV, crystal strain causes dislocations and increases elongation to break compared to PHB. The isomorphism also favors single-crystal formation, making the material an interesting candidate for studies on single-crystal formation and properties of copolymers.

Single crystals of PHB were first reported over 30 years ago,[8] for polymer as isolated from *Bacillus cereus*. Since then, single crystals of PHB have been prepared from chloroform/ethanol,[9,10] propylene carbonate,[11−16] poly(ethylene glycol),[17,18] toluene,[18] octanol,[18] and glycerol triacetate.[19] Investigations have been made of the effects of temperature,[11,12] solvent,[16] and varying amounts of HV in PHBV copolymers,[12,16,20,21] including the PHV homopolymer.[21,22] The chain-folding behavior in these single crystals has been investigated by selective degradation by methylamine[13,15] and lithium amide bases,[14] and single crystals have been prepared from natural[10] and chiral synthetic[14] oligomers of the polymer to explore the properties of the single-crystal structure in the absence of chain folds. With the exception of the latter, all of these studies have focused on the bacterial polymer, with its completely isotactic, *all-R* structure.

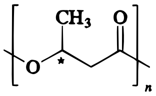

Figure 1. Poly(β-hydroxybutyrate).

In parallel with the investigation of bacterial PHA's, effort has also been directed to the preparation of synthetic equivalents. Ring-opening polymerization of racemic β-butyrolactone yields crystalline racemic PHB, the crystalline structure of which is identical by X-ray diffraction with that of fully isotactic polymer isolated from bacteria.[23] The crude product of such reactions can be fractionated to give material of varying tacticities, with varying molecular weights and levels of crystallinity.[24−26] Until recently, the tacticity range of such fractions was limited to varying degrees of isotactic material; the wide-angle X-ray diffraction patterns of such materials showed the same pattern as observed for bacterial PHB but with evidence of sharper reflections with increasing isotacticity.[26] Passing mention has been made of single crystals of synthetic racemic PHB prepared from the most isotactic fraction,[27] but no thorough investigation of the single-crystal morphology of racemic PHB has been reported. Single crystals of synthetic racemic PHV were recently reported[21] and described as less regular than those of bacterial PHV.

Recently, synthetic syndiotactic PHB has also been prepared, both through use of new stannoxane catalysts which give a preferentially syndiotactic placement when used with racemic monomer[28−32] and as a trace fraction using the same aluminoxane catalysts developed for isotactic polymer.[33] Although this material cannot yet be prepared to the same high levels of stereoregularity obtained for isotactic polymer (70% syndiotactic diads, versus 85−90% isotactic diads), it is sufficiently crystalline that a wide-angle X-ray diffraction pattern distinctly different from that of isotactic PHB can be recorded. Crystallization kinetics from the melt were found[34] to be similar to those of natural PHB. Several studies have examined the enzymatic degradability of synthetic PHB as a function of tacticity[31,35−39] but have been unable to separate tacticity effects from crystallinity effects. If crystallinity effects can be normalized by using lamellar substrates from material of widely differing stereoregularity, tacticity effects should be more clearly identifiable.

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In this study, the feasibility of preparing single crystals of synthetic racemic PHB of the full range of tacticities, from highly isotactic through essentially atactic to predominantly syndiotactic, was examined. The ethanol/chloroform solvent system was chosen to allow continuous adjustment of the precipitating solvent to compensate for the variations in solubility of the different fractions. The resulting morphologies were examined by transmission electron microscopy. Enzymatic degradation of these materials is discussed in the following paper.

Experimental Section

Polymer Synthesis. Polymer samples were obtained by bulk ring-opening polymerization of racemic β-butyrolactone with a preformed methylaluminoxane catalyst, using vacuum techniques. The isolated polymer was fractionated by solubility in chloroform/methanol and in acetone at different temperatures, yielding fractions ranging from primarily syndiotactic to highly isotactic. Tacticities were determined from the percent isotactic diads, measured by proton-decoupled ^{13}C NMR, where 50% isotactic diads refers to completely atactic material. Molecular weights were determined by gel permeation chromatography (GPC), with universal calibration using polystyrene standards. Thermal properties were measured by differential scanning calorimetry (DSC) calibrated with indium and isodecane, at a heating rate of 20 °C/min. Melting temperatures and enthalpies of fusion were from the first DSC scan, and the peak maximum of the highest melting endotherm was used if multiple endotherms were present; glass transition temperatures were from a second scan after quenching at 200 °C/min. Complete details of the polymerization, fractionation, and characterization have been previously published.[33]

Single-Crystal Preparation. Single crystals were grown by a method modified from that of Marchessault *et al.*[10] A measured amount (4−15 mg) of polymer was dissolved in chloroform, to which was added warmed ethanol to give an ethanol/chloroform ratio ranging from 2:1 to 5:1, at a fixed polymer concentration of 0.025%, w/v. The solution was then held at 60 °C (for highly isotactic PHB) or 40 °C (for slightly isotactic or syndiotactic PHB) for 7−24 h, slowly cooled to room temperature, and then reheated to 60−80 °C to dissolve the precipitate and permit self-seeding. The solution was again held at 40 or 60 °C and then slowly cooled to room temperature. Crystals were isolated by centrifugation, rinsed with room temperature ethanol to avoid contamination by unprecipitated polymer, and then stored in ethanol in the refrigerator until electron microscopy could be performed.

Transmission Electron Microscopy. Drops of the PHB suspension were deposited on 400-mesh electron microscope grids covered with a 10 nm thick carbon film. The specimens were allowed to dry and subsequently examined in a Philips EM 400 T electron microscope. This instrument was operated at an accelerating voltage of 120 kV and equipped with a low-dose unit. Electron micrographs were recorded by diffraction contrast in the bright field mode from never-irradiated areas of the grid at a plate magnification of 6000x with underfocusing conditions of 200−1000 nm. Electron diffraction patterns were recorded from selected areas 0.5 or 1.0 μm in diameter. Gold was evaporated onto some of the specimens for the calibration of the *d*-spacings.

Results and Discussion

Characteristics of the PHB Samples. The physical characteristics of the samples used in this study are reported in Table 1. The synthetic samples were the fractionated products of polymerizations with a single methylaluminoxane catalyst;[33] the tacticities vary from highly isotactic (88% isotactic diads) through slightly isotactic/atactic (60% isotactic diads) to predominantly syndiotactic (34% isotactic diads). Average isotactic ($\bar{n}_i$) and syndiotactic ($\bar{n}_s$) block lengths were calculated[40] for each of these samples using diad and triad intensities measured by high-resolution ^{13}C NMR; this study also predicted the presence of a small number of long isotactic sequences in this syndiotactic material. The glass transition temperatures (T_g's) of these samples are all similar to that of bacterial PHB; the melting temperature (T_m) of highly isotactic synthetic PHB is also similar to that of bacterial PHB, while those of the slightly isotactic and syndiotactic samples are substantially lower. The variation in the heats of fusion (ΔH_f's) of these samples corresponds to the variations in tacticity, indicating that the slightly isotactic material is close to amorphous while the highly isotactic and syndiotactic samples have substantial crystallinity.

Table 1. Characteristics of the PHB Samples Used To Prepare Single Crystals

PHB sample	isotactic diads (%)	$\bar{n}_i$[a]	$\bar{n}_s$[a]	M_w (g/mol)	T_g (°C)	T_m (°C)	ΔH_f (J/g)
bacterial	100	∞	0	500 000	4	173	85
highly isotactic	88	16	3	190 000	5	164	65
slightly isotactic	60	4	3	65 000	8	98	6
syndiotactic	34	4	5	2700	4	84	37

[a] Calculated as in ref 40; listed as number of hydroxybutrate units.

Table 2. Optimal Solvent Compositions for the Morphological Examination of Bacterial and Fractionated Synthetic PHB

PHB sample	optimal EtOH:CHCl$_3$	precipitated yield obtained (%)
bacterial	2:1	95
highly isotactic	2.5:1	80
slightly isotactic	3:1	55
syndiotactic	5:1	30

Morphologies of the Precipitated PHB. As the synthetic materials, particularly those with a low percentage of isotactic diads, are more soluble than bacterial PHB, an increased proportion of ethanol is required to achieve precipitation. The optimal ethanol/chloroform ratio to precipitate each material was determined by preparing a series of solutions at a constant polymer concentration of 0.025%, w/v, varying only the solvent composition; the resulting crystals were isolated, evaporated to dryness, and weighed to determine the precipitated yield. The point at which the precipitated yield did not significantly increase with an increase in the proportion of ethanol was taken as the optimal ethanol/chloroform ratio (Table 2). As expected from the polymer fractionation, increasing proportions of ethanol were required to precipitate the samples as the isotacticity decreased. For bacterial and highly isotactic synthetic PHB, almost quantitative precipitation could be obtained; for slightly isotactic and syndiotactic material, the precipitated yields were substantially lower and could not be significantly increased even by adding a large excess of ethanol.

As previously shown,[27] the highly isotactic polymer gave single crystals (Figure 2) virtually identical with those observed for the bacterial polymer.[8−20] As with bacterial PHB, most of the polymer crystallizes as uniform lamellae which cluster to form large aggregates. The lamellae have the same spear or lath shape as described for bacterial PHB. Lamellar dimensions varied from 0.25 × 5 μm long, shown in the micrograph, to 1 × 5−50 μm in other areas of the grid; the larger dimensions also correspond to those previously reported[17,18] for single crystals of bacterial PHB. Electron diffractograms of the lamellae display the same sharp diffraction spots as observed for base-plane diffraction of crystalline bacterial PHB.[10−12,17−19] The overlap of other crystals of slightly different orientation is respon-

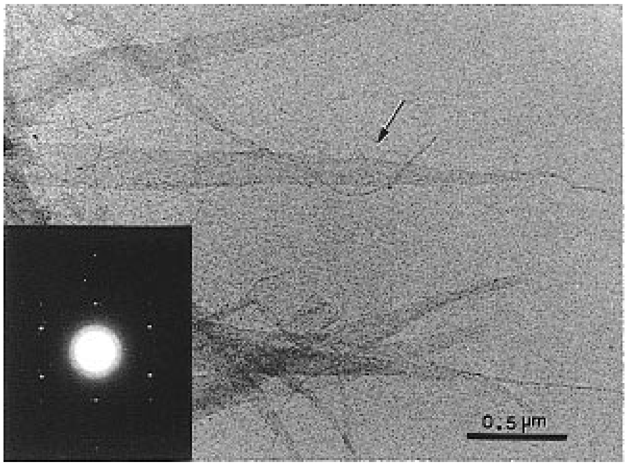

Figure 2. Transmission electron micrograph of single crystals of highly isotactic racemic PHB, grown in 2.5:1 ethanol/chloroform. Inset shows the electron diffractogram recorded from an area of the crystal such as that indicated by the arrow.

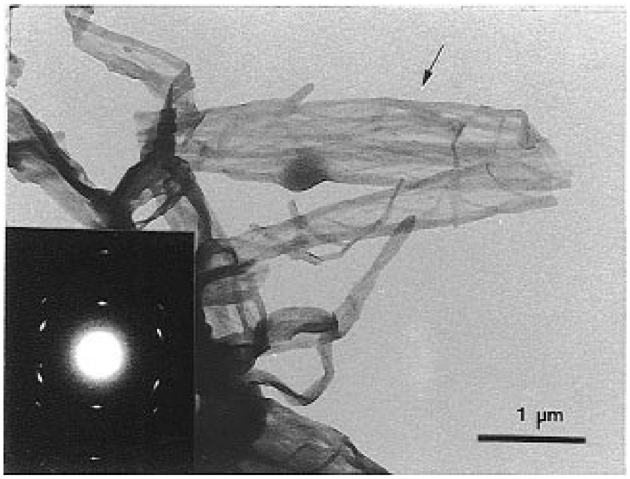

Figure 3. Transmission electron micrograph of single crystals of slightly isotactic racemic PHB, grown in 3:1 ethanol/chloroform. Inset shows the electron diffractogram recorded from an area of the crystal such as that indicated by the arrow.

Figure 4. Transmission electron micrograph of "single crystals" of syndiotactic PHB, grown in 5:1 ethanol/chloroform.

sible for the satellite reflections in Figure 2. Calculation of the average isotactic sequence length for this sample as 16 hydroxybutyrate units[40] corresponds to a distance along the helical chain of 48 Å; assuming that the lamellar thickness is $\lesssim$40−65 Å measured for single crystals of bacterial PHB,[10,11,14,15,17,18] this allows the possibility for each chain stem to contain a single isotactic sequence, with the short syndiotactic sequences also detected by NMR accommodated in the chain folds.

The essentially atactic sample forms a lamellar structure (Figure 3) similar to that of isotactic PHB, though apparently denser, with wrinkling and twisting. Electron diffraction on these lamellae yields the same pattern as isotactic PHB; a greater degree of crystal imperfection results in broadening and slight arcing of the diffraction spots. Despite the low overall tacticity of this material, sufficiently isotactic sequences are present to create the same fold structure and helix organization as in isotactic PHB. However, formation of the fold structure is more difficult for the lower tacticity material, as suggested by the longer precipitation time and the requirement of a thermodynamically poorer solvent. Similar decreases in the rate of single-crystal formation for PHBV relative to PHB led to the hypothesis that the HV component tends to concentrate in the chain folds and thus be excluded from the lamellar core,[12] a process which would require additional time for organization and result in a more irregular and probably thicker fold surface. A similar phenomenon could be occurring for the essentially atactic PHB, with the sequences of lower isotacticity congregating in the chain folds. Alternatively, if the nearly atactic sample contains separate chains of higher and lower tacticity, the higher tacticity chains could organize into single crystals while the lower tacticity ones remain in solution. NMR experiments to distinguish between these two possibilities are reported in the following section.

The discovery that single crystals of a structure similar to those of isotactic PHB can be grown from essentially atactic material suggests that the preference for the isotactic crystal structure is stronger in PHB than in other polymers which have been studied at varying tacticities. In a study of solution-grown crystals from fractions of isotactic polypropylene,[41] the lack of structural regularity was found to severely disrupt single-crystal formation even at 82% isotactic diads, the lowest tacticity examined in the study. When similar crystals were grown from syndiotactic polypropylene of tacticities varying from highly syndiotactic to nearly atactic,[42] the single-crystal perfection dropped off sharply for the more atactic material; electron diffraction has been reported only for highly syndiotactic polypropylene with 77%,[43] 94%,[44] and >99%[45] syndiotactic diads. In the case of atactic polystyrene, the authors of one study were sufficiently confident of the inability of the material to crystallize that atactic polystyrene was used as the solvent for growth of single crystals of isotactic polymer.[46]

A morphology somewhat different from that of single crystals of isotactic PHB is observed for syndiotactic PHB. The precipitate obtained using the solvent composition given in Table 2 (Figure 4) contains some structures similar to those seen for slightly isotactic PHB but also a large quantity of less well-defined globular structures, 0.1−0.4 μm in diameter, presumed to be hedrites. Electron diffractograms could not be recorded for either of these structures, as they are so thick that they were essentially opaque to the electron beam. A lamellar structure similar to that of isotactic PHB was observed for syndiotactic PHB precipitated in very low (<1%) yield from 3:1 ethanol/chloroform (Figure 5). Because these structures were obtained in such small yield and precipitated much more slowly, they are larger than many of those observed in isotactic PHB (1 μm wide by 10 μm long); however, there is still a

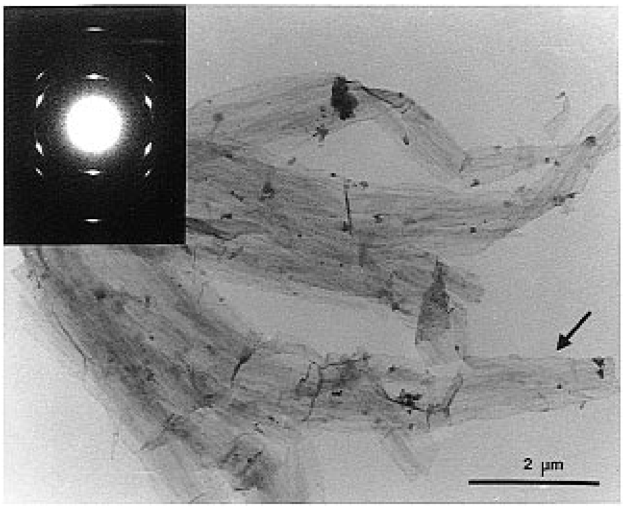

Figure 5. Transmission electron micrograph of single crystals of syndiotactic PHB, grown in 3:1 ethanol/chloroform. Inset shows the electron diffractogram recorded from an area of the crystal such as that indicated by the arrow.

Table 3. Tacticity Changes with Lamellar Single-Crystal Formation in Synthetic Racemic PHB of Varying Tacticity

	isotactic diads (%)		
PHB sample	original	in crystals	in supernatant
highly isotactic	88[a]	89	[b]
slightly isotactic	60	62	58
syndiotactic	34	40	39

[a] All measurements are ±5%. [b] Insufficient unprecipitated polymer to measure.

tendency for the lamellae to aggregate. Diffraction could be recorded for this lamellar precipitate; surprisingly, the diffractogram for this material is identical with that of isotactic PHB, despite significant differences in the wide-angle X-ray scattering by isotactic and syndiotactic crystal structure.[28,31−33] This indication that the low-yield precipitate is primarily isotactic implies that segregation is occurring during the precipitation procedure.

Fractionation in the Precipitated PHB. To examine fractionation according to tacticity in the synthetic PHB samples, a set of crystals prepared using the solvent compositions given in Table 2 was characterized by ^{13}C NMR. The precipitate in each case was isolated by centrifugation, washed with ethanol, and then evaporated to dryness and dissolved in $CDCl_3$ for determination of the percentage of isotactic diads by ^{13}C NMR; for those samples where significant soluble material remained, the supernatant was also evaporated and examined by ^{13}C NMR. The high precipitated yield of the most isotactic sample left insufficient soluble material for NMR examination; this was not of major concern as the well-defined spectrum for the isotactic single crystals agreed well with that of the original sample. More significantly, no spectral examination could be performed for syndiotactic PHB precipitated in 3:1 ethanol/chloroform (Figure 5), as the precipitated yield in this case was so small that the total precipitated mass would not be adequate to provide an observable and quantifiable ^{13}C NMR signal; similarly, the absence of so small an amount of material would not cause a significant change in the tacticity of the polymer remaining in solution.

Results of this NMR experiment are shown in Table 3 and indicate that under the precipitation conditions used, minimal fractionation occurs. Since the slightly isotactic material shows no significant separation into highly isotactic crystals and atactic solution, the less isotactic material must be somehow incorporated into the crystals; presumably, the least isotactic portions of the chains are accumulated in the chain folds, as previously suggested. In the syndiotactic material, Figure 4 clearly shows two precipitated structures; since the dominant form under less favorable precipitating conditions (Figure 5) is the lamellar one which shows isotactic diffraction, it seems likely that the lamellar structures contain the more isotactic chains while more syndiotactic chains are localized in the globular structures. Thus, the overall precipitated tacticity could remain the same as that remaining in solution, despite segregation within the precipitated material.

Without NMR data for the syndiotactic PHB under only slightly precipitating conditions, one can only hypothesize about the possibility of fractionation in this case. Given that the syndiotactic material was obtained by fractionation from a much larger amount of isotactic PHB, it is quite possible that this material contains a small number of isotactic chains or parts of chains, in addition to a much larger number of syndiotactic chains. As isotactic PHB crystallizes relatively fast, it is likely that under weakly precipitating conditions, only this isotactic contaminant can precipitate and crystallize. Syndiotactic material in the same chains as isotactic blocks could then accumulate in the chain folds in a manner similar to that envisioned for the slightly isotactic material, or if the small isotactic component is primarily localized in separate chains, the predominant syndiotactic component could simply remain in solution. The low crystallization yield is consistent with the presumption of precipitation of a trace contaminant. Recent investigation into the microstructure of the fractionated polymer by high-resolution ^{13}C NMR showed that the polymerization mechanism which best fits the NMR intensities implies the presence of a small number of highly isotactic chains in the syndiotactic material.[40] The discovery that trace amounts of isotactic crystals can be fractionally crystallized from predominantly syndiotactic material provides additional support for this model.

The fact that single crystals of the essentially atactic material could be grown without overall fractionation of the sample provides evidence that cocrystallization of the *R* and *S* units can occur to some extent, as was previously suggested.[24,47] The average isotactic chain length of only four repeat units in this material[40] corresponds to a distance along the helical chain of only 12 Å, which is significantly smaller than the assumed stem length or distance along the chain from one fold to the next. Thus, even if the most irregular sequences are accumulated in the chain folds, occasional asymmetric carbons of the opposite configuration must be present in the lamellar core. As the intramolecular dipole interactions responsible for stabilizing the isotactic PHB helix involve the planar or near-planar ester groups,[48−50] an occasional change in configuration at the asymmetric carbon would not necessarily destabilize the helical structure. Preliminary molecular modeling results[51] suggest that relatively modest changes in bond angle at a single ester group would relieve the overlap of the methyl and carbonyl groups for an *R*-substituted carbon in a right-handed helix or an *S*-substituted carbon in a left-handed helix. Apparently, the energy cost of such deviation at an occasional carbon of the "wrong" configuration is more than compensated by the favorable energies of crystallization.

Conclusions

Ths study has shown that single crystals of PHB can be prepared from synthetic racemic PHB of varying tacticities. This is possible even for material which is close to atactic in overall composition; the proportion of the material which precipitates in such cases is lower, and the precipitation time is longer. The morphology and electron diffraction of single crystals obtained from PHB fractions which are only slightly isotactic are similar to that previously observed for bacterial PHB. As the overall tacticity of the essentially atactic single crystals was not significantly affected by the precipitation, this implies that some degree of cocrystallization of the *R* and *S* repeating units can occur.

Crystalline lamellar precipitate can also be obtained from syndiotactic PHB; if this material has been obtained by fractionation from more isotactic polymer, lamellar structures similar to those of isotactic PHB are observed, as well as globular hedrites. Diffraction of lamellar crystals obtained from such material under conditions of weak supersaturation show the typical diffractogram of isotactic PHB; it is therefore presumed that the lamellae contain more isotactic material while the hedrites contain more syndiotactic chains. Determination of the overall tacticities of both precipitated and nonprecipitated polymer by ^{13}C NMR indicates that little overall fractionation occurs under conditions which precipitate measurable quantities of polymer. Thus, the results of enzymatic degradation experiments using these precipitates could be interpreted in terms of the tacticities of the original material.

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(51) Unpublished data from Dr. Stephane Raymond (Chemistry Department, McGill University), who minimized chains of 100 β-hydroxybutyrate units with varying proportions of random *R* and *S* centers.

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