Synthesis and Properties of Hydroxy-Terminated Poly(hydroxyalkanoate)s

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ABSTRACT: This paper describes the biosynthesis and properties of bacterial poly(hydroxyalkanoate)s (PHA) with predominantly hydroxyl end groups. Hydroxy termination is achieved by the addition of low molecular weight diols to the culture. It is found that low molecular weight diols of various structures can be easily incorporated as chain ends, when used during fermentation, by a variety of microorganisms. Incorporation of a chiral diol does not appear to be stereospecific; both (*R*)- and (*S*)-1,2-propanediols were incorporated into the polymer. Moreover, both primary and secondary hydroxyl groups of 1,2-propanediol were found to have reacted. It was found that an increase in the hydroxy termination in P(3HB) leads to an increase in the thermal stability, most likely by prolonging the condensation reaction and delaying the degradation reaction.

Introduction

Poly(hydroxyalkanoate)s [PHAs] are a group of storage polymers produced by many bacteria in response to growth restriction by a nutrient other than the carbon source.[1] PHAs are generally biodegradable plastics and possess properties ranging from those of elastomers to thermoplastics and thermosets. Poly(3-hydroxybutyrate) [P(3HB)] was the first example of a PHA to be discovered.[2] The physical and mechanical properties of PHAs are determined by composition and monomer sequence. Attempts at improving the properties of PHAs have focused on incorporation of different monomers,[3−7] altering the chain alignment within the polymer,[8] introducing branching,[9] and blending of the PHA with other polymers.[10,11] For example, the properties of P(3HB) can be altered by copolymerization with other monomers. Copolymers of 3-hydroxybutyrate [3HB] (>70 mol %) and 3-hydroxyvalerate [3HV] have been produced on a commercial scale. The thermal and mechanical properties of P(3HB), for example, are favorably changed by copolymerization with 3HV.

Recently, additional chemical and biological routes to synthesizing novel block copolymers based on PHAs have been reported. Telechelic P(3HB) of low weight-average molecular mass [M_w] ($(1.5-2.0) \times 10^3$ g mol^{-1}) with hydroxy functionalities at each end has been prepared by a transesterification reaction of ethylene glycol [EG] and bacterial P(3HB).[12a] These telechelics have then been employed in the synthesis of di- and multiblock copolymers.[12b] Diblock copolymers have also been produced by the use of poly(ethylene glycol) during fermentation.[13]

Control of polymer end group is critical for several end-use applications. Poly(oxymethylene) and some polyesters, for example, are functionalized at the chain ends to stop the degradation reactions that take place by backbiting mechanisms. As mentioned earlier, functionalized chain ends are also useful for carrying out further reactions, for example, to produce block copolymers.

The authors have previously reported the biosynthesis of P(3HB) with the desired end functionalization incorporated by supplementation of the culture medium with various alcohols, diols, and polyols.[14] A carboxyl group belonging to the final 3HB monomer in a P(3HB) chain is normally found at the chain terminus.[14] However, this has been altered in vivo by the inclusion of hydroxy-containing compounds, which become incorporated at the polymer terminus via a chain-transfer reaction in which the growing P(3HB) chain is esterified to the hydroxy compound.[14] This technique has been used to produce hydroxy-terminated P(3HB)[14,15] and block copolymers.[13,16]

Thermal degradation of P(3HB) occurs quite quickly at temperatures slightly higher than the melting temperature (T_m) of the polymer (171 °C).[17] Copolymerization with 3HV is used as a route to lower the melting point of the polymer so that thermal processing can be achieved. The T_m of the copolymer decreases as the 3HV content is increased. This has been the basis for the commercial-scale production of Biopol, a copolymer of 3HB and 3HV, which is melt processable because the T_m is considerably below the degradation temperature.

As thermal degradation of PHAs takes place via random chain scission,[17] traditional thermal stabilizers are not effective. During thermal processing of PHAs, both chain-end condensation, leading to an increase in molecular weight, and chain scission, leading to a decrease in molecular weight, take place.[18] Since each chain scission event produces a carboxyl and a crotonate chain end, the carboxyl concentration increases considerably with time, and chain end condensations are minimized, leading to a drastic reduction in the molecular weight.[17] The approach taken in the present study is to increase the available hydroxyl chain-end concentration so that condensation reactions are prolonged and

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the overall degradation of the polymers is slowed down.

This paper describes the production of hydroxy-terminated PHAs by fermentation using ethylene glycol and propylene glycol as chain-transfer agents. The effect of hydroxyl end groups on the molecular weight and thermal stability of the polymers is also reported.

Experimental Section

Materials. Unless specified, all reagents were purchased from Aldrich Chemicals, USA, and solvents were from VWR Scientific Products. The organic components of all fermentation media were from Difco, and the inorganic media components were purchased from Sigma.

Production of Hydroxy-Terminated PHA in Shake-Flask Culture. *Ralstonia eutropha* (reclassified from *Alcaligenes eutrophus*) strain NCIMB 40529 was grown in 250 mL shake flasks containing 50 mL of LB medium (LB medium contains 10 g L^{-1} of typtone (Difco), 5 g L^{-1} of yeast extract (Difco), and 5 g L^{-1} of NaCl) plus glucose (2%) and ethylene glycol (EG) or propylene glycol (1,2-propanediol, PG) at levels ranging from 1% to 5%. The culture flasks were incubated at 30 °C for 4 days in a shaker (New Brunswick) orbiting at 250 rpm to obtain hydroxy-terminated P(3HB). Ethylene glycol (EG) was also used to produce hydroxy-terminated P(3HB-*co*-4HB) in 50 mL of a minimal salt medium plus 2% glucose and 0.5% sodium 4-hydroxybutyrate. Bacteria were grown at 30 °C for 4 days.

Comamonas testosteroni was grown in 250 mL shake flasks containing 50 mL of LB medium plus 2% oleic acid and 5% ethylene glycol (EG). The culture flasks were incubated at 30 °C for 3 days in a shaker (New Brunswick) orbiting at 250 rpm. Cells were harvested, dried, and extracted with chloroform at 100 °C for 2 h.

Production of Hydroxy-Terminated P(3HB) via Fed-Batch Fermentation. *R. eutropha* was grown in a minimal medium (50 mL) with glucose (2%) in a shake flask overnight, and the whole culture was used to inoculate a Braun Biostat B fermenter with a 2 L vessel. The initial volume of the medium was 1 L. The medium at the start of fermentation consisted of the following (g L^{-1}): glucose (20), diol (% specified), $(NH_4)_2SO_4$ (4), $MgSO_4 \cdot 7H_2O$ (2.2), citric acid (1.7), and trace elements (8 mL). The trace element solution consisted of the following (g L^{-1}): $FeSO_4 \cdot 7H_2O$ (10), $ZnSO_4 \cdot 7H_2O$ (2.25), $CuSO_4 \cdot 5H_2O$ (0.5), $CaCl_2 \cdot 2H_2O$ (2), H_3BO_3 (0.1), $(NH_4)_6Mo_4O_{24}$ (0.1), HCl (10 mL).

After autoclaving, a filter sterilized solution of KH_2PO_4 (3.5 g in 30 mL of water) was added, and the pH was adjusted to 6.8 with ammonia. During the fermentation the pH was maintained at 6.8 with ammonia and 20% H_2SO_4. The fermenter was maintained at 34 °C, and the medium agitated at 730−1200 rpm with dissolved oxygen concentration maintained at a minimum of 20%. The aeration was at 4 L/min. When the glucose in the medium was exhausted (determined by YSI 2700 Select glucose analyzer, YSI, Inc. Yellow Springs, OH), feed consisting of 0.5 L of water, 400 g of glucose, and the corresponding percentage of diol was introduced to the vessel. The feeding rate was varied depending on the rate of consumption of glucose, such that the glucose concentration was maintained at less than 2%. During the fermentation, foam was controlled by 50% SAG471 (OSI Specialties, Sistersville, WV). The fermentation run was continued until the feed was exhausted.

The following diols were included in the feed during separate fermentation runs that were carried out as described above: 1−5% ethylene glycol, 1−5% propylene glycol (1,2-propanediol).

Procedure for Isothermal Thermogravimetric Analysis (TGA). The samples, in powder form, were tested by simultaneous DTA-TGA using an Omnitherm STA 1500 thermogravimetric analyzer and analyzed using the accompanying software package. The instrument weight axis was calibrated by a calibration weight according to the instrument SOP. The TGA module measures weight loss as a function of temperature through the use of a null seeking balance. The furnace was calibrated using a number of temperature stability readings at 2 °C intervals. The procedure followed in the analysis involved heating a series of four, 5 ± 1 mg, samples isothermally at 200 °C. The samples were heated from 25 °C and maintained at 200 °C for 120 min, and the weight lost over time was recorded.

Procedure To Determine the Activation Energy of Thermal Degradation of PHA Samples by TGA. The samples, in powder form, were tested by simultaneous DTA-TGA as described above. The procedure followed in the analysis was based on the ASTM Standard E 1641-94 ("Standard Test Method for Decomposition Kinetics by Thermogravimetry") and involved heating a series of four samples (5 ± 1 mg) at different heating rates, specifically 1, 2, 5, and 10 K/min. The samples were heated from 25 °C through their decomposition (from 230 to 310 °C). The temperature at which the sample lost 10% of its weight was recorded for the four rates. An Arrhenius plot [log(heating rate (K/min)) vs 1/temperature (K)] was obtained, and the slope was determined by least-squares fit. The slope was then used in the calculations to determine the activation energy.

Procedure To Determine Molecular Weight (M_n). Quantitative end group analysis entailed derivatization of the hydroxyl and carboxyl ends of PHAs with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (CTMDP) and integration of the resulting ^{31}P NMR spectrum. The ^{31}P chemical shifts of the hydroxyl-terminated ends ranged from 145 to 150 ppm, while those of the carboxyl-terminated ends did not deviate significantly from 135.4 ppm. A measured amount of Bisphenol A (which was also derivatized in the process) added to the reaction mixture provided a chemical shift reference (138.38 ppm) and a quantitative reference from which the absolute number of end groups per polymer chain, and therefrom the number-average molecular weight (M_n), could be calculated. Further details of the procedure are described in the literature.[9] Chromium acetyl acetonate was added before acquisition of the spectrum in order to justify an acceptably short pulse repetition rate (5−7 s; T_1 without relaxation reagent ∼20 s). From experiment to experiment the ratio of reagents was kept as constant as possible in order to avoid fluctuations in the ionicity of the NMR sample which affected the length of the 90° pulse (which had to be specifically calibrated for this experiment).

Absolute weight- and number-average molecular weights were determined by gel permeation chromatography (GPC) on Waters 150CV system with 3 PLGel columns (Polymer Laboratories) of 10 μm particle size connected in series and a dual detection system consisting of a differential refractometer and a single capillary viscometer in series. Chloroform was the eluent at a flow rate of 1.0 mL/min, sample concentrations were typically 2 mg/mL, and the injection volume was 300 μL. A stock solution of chloroform containing 1,2,4-trichlorobenzene (350 μL in 430 mL of chloroform) as a flow rate marker was used to prepare the samples. The temperature of the samples, columns, and detectors was maintained at 35 °C. Narrow polydispersity polystyrene standards (Toyo Soda, Japan) were used to generate a universal calibration curve, from which the molecular weights were determined after correcting for flow rate variations based on the elution volume of the flow rate marker.

^{13}C NMR spectra suitable for integration were acquired on a 400 MHz Varian Unity NMR spectrometer operating at a ^{13}C frequency of 100.574 MHz, using a 90° pulse, 16 s delay, and gated decoupling.

Results and Discussion

Production of Hydroxyethyl-Terminated P(3HB) and P(3HB-*co*-4HB) Using Ethylene Glycol. Microbial synthesis of PHAs has been carried out with *R. eutropha* in the presence of several diols.[13−16] However, the simplest diol, ethylene glycol [EG], has not been utilized previously, presumably due to the expected toxicity of this compound in vivo. In this work, in an

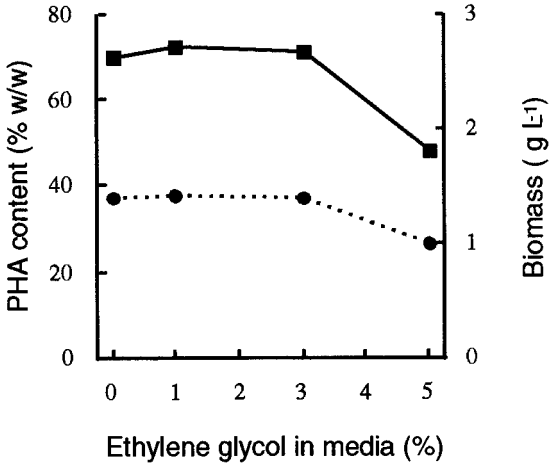

Figure 1. Effect of ethylene glycol on polymer, P(3HB-*co*-4HB), content (■) and biomass concentration (●) in shake-flask culture.

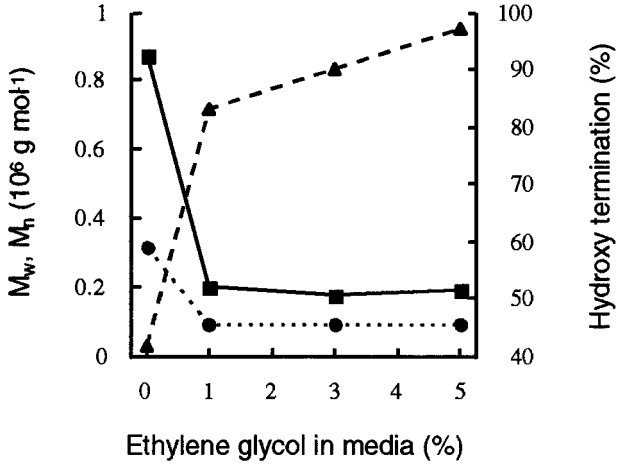

Figure 2. Effect of ethylene glycol on polymer, P(3HB-*co*-4HB), M_w (■), M_n (●), and percentage of hydroxy-terminated polymer chains (▲) in shake-flask culture.

effort to obtain hydroxy-terminated PHAs, fermentation were carried out in the presence of EG in the culture media. *R. eutropha* was used as the bacterial strain for most of the work described here since this strain is known to produce polymers of high molecular weight, and it has been utilized to produce PHAs at commercial scale. PHA molecular weight could be influenced by the appropriate choice of bacterial strain and the carbon source.[15] To produce PHA of relatively lower molecular weight, a shake-flask experiment was carried out using *C. testosteroni* with oleic acid as the carbon source. For the work described here hydroxyethyl-terminated P(3HB-*co*-4HB) and P(3HB) were synthesized and evaluated.

Hydroxyethyl-Terminated P(3HB-*co*-4HB). Hydroxyethyl-terminated P(3HB-*co*-4HB) was produced using glucose and sodium 4-hydroxybutyrate as carbon sources in the presence of EG. The fermentation conditions, amount of glucose, and sodium-4-hydroxy butyrate in the feed were kept constant, and the amount of EG was changed from 0 to 5%. The amount of 4HB incorporated in these polymers ranged from 13 to 15 mol %, and the concentration of EG did not significantly affect the composition of the copolymer. The polymers produced in these experiments were characterized by ^{13}C NMR and found to be random copolymers of 3HB and 4HB monomers similar to those reported by Saito and Doi.[3] The randomness was verified by comparing the experimental peak intensities of the assigned carboxyl peaks (which reflect compositional dyad to tetrads) with peak intensities calculated for Bernoullian statistics. Refinement of the random model by using first-order Markovian statistics did not result in an improved fit of the data. The effect of EG on biomass, P(3HB-*co*-4HB) content, and molecular weight was assessed in shake-flask culture. The biomass and PHA content of bacteria were unaffected up to 3% EG (Figure 1). However, a concentration of 5% EG resulted in a decrease in both biomass and PHA content, presumably, because at this level, ethylene glycol was toxic to the bacteria. The effect of EG on the molecular weight and percent hydroxy termination is shown in Figure 2. Considerable reduction in molecular weight and corresponding increase in hydroxyl chain ends of P(3HB-*co*-4HB) is achieved at 1% diol concentration. This indicates that EG is acting as a chain terminator.

Hydroxyethyl-Terminated P(3HB). Hydroxyethyl-terminated P(3HB) was produced by fermentation using *R. eutropha* or *C. testosteroni* in the presence of EG in the culture medium. Glucose and oleic acid were utilized as carbon sources in fermentations carried out using *R. eutropha* and *C. testosteroni*, respectively. Hydroxyethyl termination was determined as described previously by ^{31}P NMR analysis of the polymer derivatized with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (CTMDP), which reacts with and bonds to any free hydroxyl and carboxyl groups in the polymer.[14,19] An example of a ^{31}P NMR spectrum of P(3HB) terminated with EG is shown in Figure 3. The ^{31}P peaks arising from deriva-

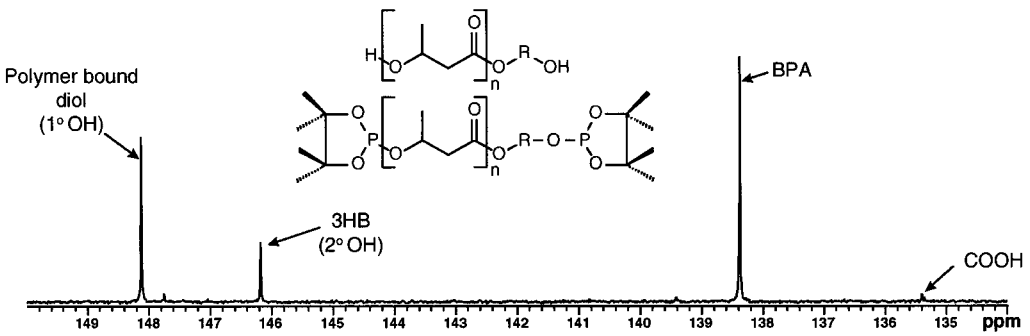

Figure 3. ^{31}P NMR spectrum of P(3HB) terminated by ethylene glycol. The peaks correspond to hydroxyl groups bonded to CTMDP. BPA (bisphenol A) is included as a reference at 138.38 ppm, 3HB is the secondary hydroxyl group from the first monomer in a P(3HB) chain, COOH is the carboxyl group at the end of a P(3HB) chain, and there is a peak for a primary hydroxyl group remaining from a P(3HB)-bound diol (R), also at the end of a polymer chain.

Table 1. Comparison of M_n Values Based on End-Group Analysis from ^{31}P NMR and GPC

% EG	fermentation conditions	M_n, from 2° OH of 3HB (^{31}P NMR)	M_n, from COOH + 1° OH of EG (^{31}P NMR)	M_n (GPC)	% OH end groups (^{31}P NMR)	% chains primed by non-OH-containing moieties
1	*R. eutropha* + glucose	108 400 (± 2198)	55 900 (±1739)	55 750 (±1485)	81	32
3	*R. eutropha* + glucose	89 100 (±2089)	47 100 (±3023)	46 350 (±71)	95	30
5	*R. eutropha* + glucose	104 200 (±2063)	37 000 (±1531)	36 950 (±1273)	96	48
5	*C. testosteroni* + oleic acid	43 800 (±765)	17 900 (±1196)	18 200 (±1485)	91	42

tized hydroxyl and carboxyl groups can be clearly identified and quantified. The peak at 148.13 ppm of the derivatized primary hydroxyl group of the polymer-bound EG is shifted downfield from that of the secondary hydroxyl group of the P(3HB) (146.19 ppm), both of which are downfield in relation to the derivatized carboxyl group peak (135.40 ppm). The presence of an ethylene glycol terminus shows that EG was involved in the chain-termination reaction.

The ease of identification of the ^{31}P peaks at the hydroxy ends of these PHAs and the sensitivity of this method are very useful in gaining further insights into the nature of their end functionalization. ^{31}P NMR of the derivatized ends has enabled their clear identification and quantification. This quantitative end group analysis was further used to determine the absolute M_n values for these polymers and compared with the true M_n values obtained by GPC, based on a universal calibration curve. Results of these analyses performed on several EG-based microbial hydroxy-terminated PHAs with different percentages of hydroxyl end groups and molecular weights and are presented in Table 1. These results can be directly compared with the M_n values determined by GPC from a universal calibration curve (Table 1, column 5). We find that the M_n's are significantly overestimated when calculated using the integral of the 2° hydroxyl group from the 3HB terminus, while there is excellent agreement with the GPC results of the M_n's calculated by integrating the 1° hydroxyl and carboxylic acid peaks. It is clear that the integral of the 2° OH group of the 3HB terminus underestimates the concentration of polymer chain ends, resulting in an overestimation of the M_n. This implies that not all chains contain a 2° hydroxyl terminus arising from 3HB but do have a carboxylate end that is either free or esterified by EG due to chain transfer. This finding supports the suggestion that the polymer chains synthesized during the initial unrestricted growth of bacteria involve PHA synthase that is activated by a primer that has no hydroxyl groups.[14] On the basis of the difference between the percentages of carboxylate ends (free and esterified) and the 3HB hydroxyl ends, the percentage of chains primed by non-hydroxy-containing moieties was also calculated (Table 1).

In an effort to determine whether chain termination is the only reaction in which EG participates and whether hydroxyethyl-terminated P(3HB) chains participate further in chain-transfer reaction, a fermentation was carried out using ^{13}C-labeled EG. It was found that EG becomes incorporated in the polymer only at the chain ends and was not found in the polymer chain other than at the chain terminus. EG, however, was found to be metabolized by *R. eutropha* and used as a carbon source for the production of P(3HB) (data not shown). The labeled carbons derived from the EG are incorporated in the polymer in pairs as C1−C2 and C3−C4 of 3HB monomer. It is proposed that mixing of the carbons derived from the glucose and ethylene glycol, in the P(3HB) biosynthesis, takes place only at the dimerization of acetyl-CoA.[20]

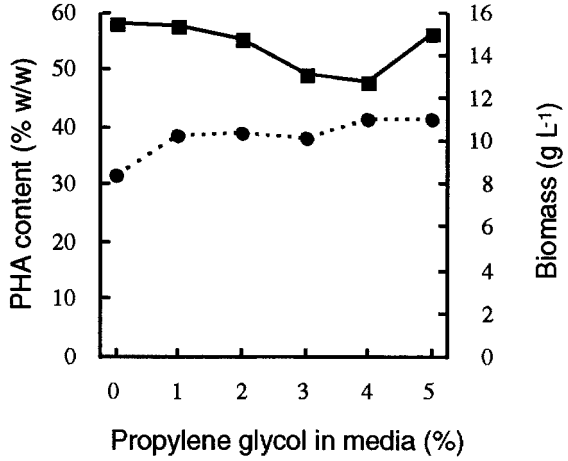

Figure 4. Effect of propylene glycol on polymer, P(3HB), content (■) and biomass concentration (●) in shake-flask culture.

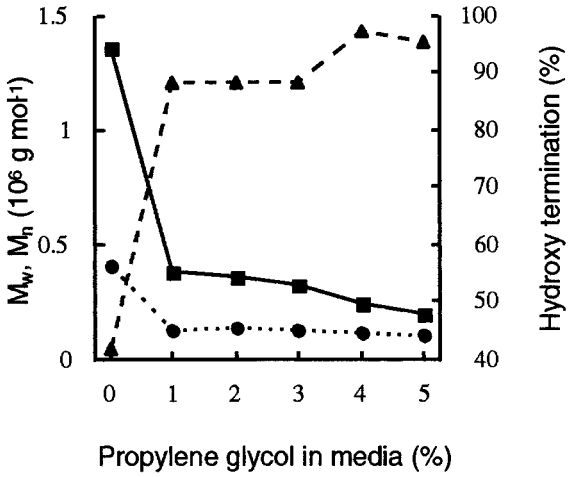

Figure 5. Effect of propylene glycol on polymer, P(3HB), M_w (■), M_n (●), and percentage of hydroxy-terminated polymer chains (▲) in shake-flask culture.

Hydroxypropyl-Terminated P(3HB) Using 1,2-Propanediol. P(3HB) containing hydroxypropyl chain ends was produced using *R. eutropha* from glucose, with propylene glycol [PG] (1,2-propanediol) in the culture medium. The effect of PG on biomass, P(3HB) content, and molecular mass of the resulting polymer was determined in shake-flask experiments. Influence of the nature and the stereochemistry of the hydroxy group on the termination reaction was also investigated. Figure 4 shows the effect of the amount of PG in the culture media on the biomass and the PHB content.

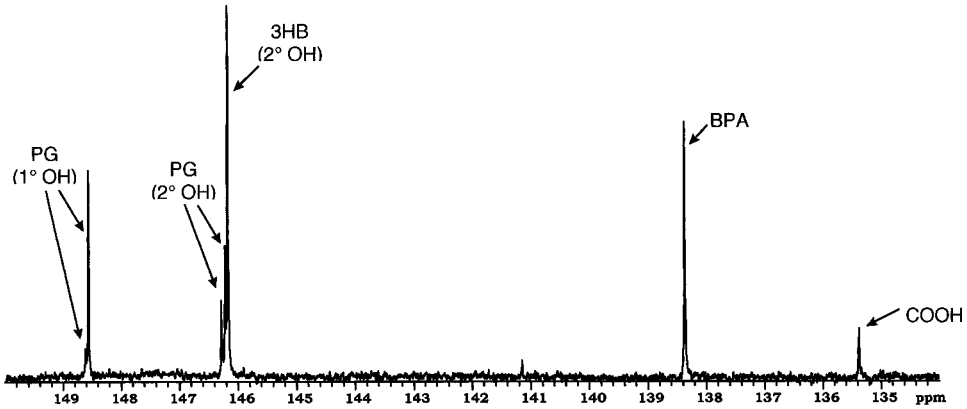

Figure 6. ^{31}P NMR spectrum of P(3HB) terminated by propylene glycol. The peaks correspond to hydroxyl groups bonded to CTMDP. BPA (bisphenol A) is included as a reference at 138.38 ppm, 3HB is the secondary hydroxyl group from the first monomer in a P(3HB) chain, and COOH is the carboxyl group at the end of a P(3HB) chain. There are four other peaks corresponding to P(3HB)-bound PG when, depending on whether the primary or secondary hydroxyl group is polymer bound, there remains either a primary or secondary hydroxyl group available to the CTMDP, and this end group can be in both (*R*) and (*S*) configurations.

Table 2. Distribution of Chain Ends in P(3HB) Produced Using Propylene Glycol as a Chain-Transfer Agent

	COOH	PHB-OH	2° PG-OH [*R*]	1° PG-OH [*R*]	2° PG-OH [*S*]	1° PG-OH [*S*]
ppm	135.39	146.18	146.23	148.58	146.29	148.62
integral	0.20	1.30	0.40	0.62	0.29	0.11
total integral				[*R*] = 1.02		[*S*] = 0.40

Neither the biomass nor P(3HB) content was affected by the concentration of PG in the medium, suggesting that PG is less toxic than EG. There was, however, a marked decrease in molecular weight and corresponding increase in the percentage of hydroxy chain ends at a concentration of 1% PG (Figure 5), similar to the effect of adding EG to the medium.

A ^{31}P NMR spectrum of the derivatized P(3HB) terminated with PG is shown in Figure 6. Four derivatized alcohol peaks, with chemical shifts 146.23, 146.29, 148.58, and 148.62 ppm, were detected in addition to the peak of the derivatized secondary alcohol end group of P(3HB) at 146.18 ppm; these were attributed to the stereoisomers, (*R*) and (*S*), of chiral PG. The two peaks at 146.23 and 146.29 ppm, adjacent to the hydroxyl of the 3HB unit, were identified as the secondary hydroxyl of a PG molecule bound to a P(3HB) chain via the primary hydroxyl of (*R*)- and (*S*)-PG. Further downfield at 148.58 and 148.62 ppm were two more peaks attributed to the primary hydroxyl of PG in both (*R*) and (*S*) configurations (Table 2). This conclusion was corroborated by the constancy of the peak integral ratio through several purification steps and by the analysis of the spectrum of PG-terminated P(3HB) prepared with (*S*)-propylene glycol. In this case we detected only two peaks originating from PG, at 146.29 and 148.70 ppm, which we assigned respectively to the derivatized secondary and primary alcohol of the (*S*) isomer of PG.

Thus, both (*R*) and (*S*) end groups are formed during chain transfer. However, it is found that chain transfer by (*R*)-PG appears to predominate over that by (*S*)-PG for both the primary and secondary hydroxyl groups (Table 2). Hence, it appears that the chirality of the chain-transfer agent may be playing a role in the chain-transfer reaction. It has been hypothesized previously[14] that, in the absence of external chain-transfer agents, (*R*)-3HB is the most probable chain-transfer agent in vivo. It is likely that the similarity in the steric configuration of the secondary hydroxyl group of (*R*)-PG to that of the hydroxyl of (*R*)-3HB enables it to preferentially access the growing end of the PHA chain covalently attached to the synthase, to cause the chain transfer. This may also explain the observation that a greater proportion of P(3HB) chains are terminated by secondary OH of (*R*)-PG than by primary OH, despite the fact that the latter might be expected to be more reactive. Although it is surprising that, in an enzyme-mediated termination, all four hydroxyls (primary and secondary of (*R*)- and (*S*)-PG) have participated in the chain-transfer reaction, differences in the reactivity among the primary and secondary hydroxyls of the two isomers is an indication that synthase may indeed be playing a role in the termination reaction. In the case of (*S*)-PG, distribution of chain transfer between the primary and secondary hydroxyls takes place on the basis of their expected reactivities.

Thermal Stability of Hydroxy-Terminated PHA. The thermal degradation of hydroxy-terminated P(3HB), produced when either EG or PG was included in the culture medium, was investigated by isothermal TGA at 200 °C and compared with that of a control sample of P(3HB). The proportion of hydroxyl end groups in the P(3HB) control, as determined by ^{31}P NMR, was found to be 40%, compared with 93% and 94% hydroxyl end groups observed for PG- and EG-terminated P(3HB), respectively. The percent weight loss with time for these polymers under isothermal TGA at 200 °C is shown in Figure 7. P(3HB) containing 40% hydroxy end groups began to lose weight after 30 min at 200 °C, and after 2 h only 15% of the original mass of the polymer is retained, the rest being converted into volatile decomposition products. In contrast, the hydroxy-terminated polymers were found to have significantly increased thermal stability, with the earliest initial weight loss recorded at 60 min, leaving a residue after 2 h that was 75−98% of the original mass. It appears that the proportion of hydroxy termination in the polymer is playing a role in determining the thermal stability of the materials. To further elucidate this, the energy of activation (E_a) for thermal degradation were determined as described earlier. It was found that while the control P(3HB) has an E_a of 98 kJ/mol, the E_a values of PG-

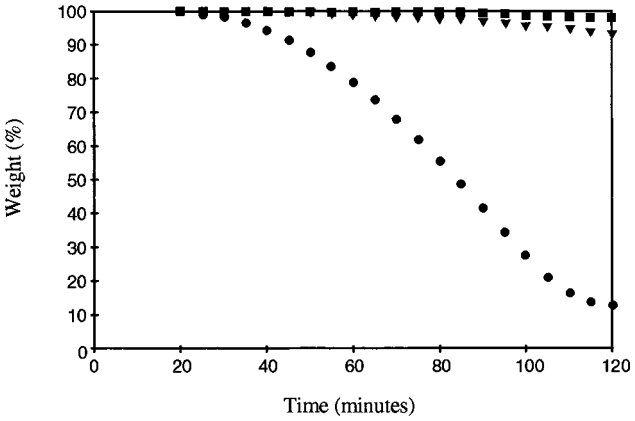

Figure 7. Percent weight loss vs time for P(3HB) [40% −OH] (●), EG-terminated P(3HB) [93% −OH] (■), and PG-terminated P(3HB) [94% −OH] (▼), as analyzed by isothermal TGA at 200 °C.

and EG-terminated P(3HB) were determined to be significantly higher at 160 and 118 kJ mol^{-1}, respectively, reflecting the increased thermal stability of the hydroxy-terminated PHAs. It is proposed that the most likely cause of this stability is a prolonging of the condensation reactions between hydroxyl and carboxyl chain ends, observed during the initial stages of thermal degradation of P(3HB),[18] and a delay before chain scission reactions dominates to cause weight loss. A single chain scission event produces a carboxyl and a crotonate end group, and if there are no hydroxyl groups for the carboxyl to react with, then the molecular mass is decreased by each event. However, if there are sufficient hydroxyl groups in the polymer, then the carboxyl group is able to react, thus countering the effect of the chain scission. This would be expected to continue much longer in hydroxy-terminated P(3HB) than in the control, before all hydroxyl groups are consumed and weight loss would be observed. A detailed study consisting of polymers with different degrees of hydroxy termination, taking into account their initial molecular weight, needs, however, to be carried out to confirm the hypothesis proposed here.

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References and Notes

(1) Dawes, E. A.; Senior, P. J. *Adv. Microbial Physiol.* **1973**, *10*, 135.
(2) Lemoigne, M. *Ann. Inst. Pasteur, Paris* **1925**, *39*, 144.
(3) Saito, Y.; Doi, Y. *Int. J. Biol. Macromol.* **1994**, *16*, 99.
(4) Shimamura, E.; Scandola, M.; Doi, Y. *Macromolecules* **1994**, *27*, 4429.
(5) Foster, L. J. R.; Zervas, S. J.; Lenz, R. W.; Fuller, R. C. *Biodegradation* **1995**, *6*, 67.
(6) Hazer, B.; Lenz, R. W.; Fuller, R. C. *Polymer* **1996**, *37*, 5951.
(7) Scholz, C.; Fuller, R. C.; Lenz, R. W. *Macromol. Chem. Phys.* **1994**, *195*, 1405.
(8) Kusaka, S.; Iwata, T.; Doi, Y. *J. Macromol. Sci., Pure Appl. Chem.* **1998**, *A35*, 319.
(9) D'Haene, P.; Remsen, E. E.; Asrar, J. *Macromolecules* **1999**, *32*, 5229.
(10) Scandola, M. *Can. J. Microbiol.* **1995**, *41* (Suppl. 1), 310.
(11) Scholz, C.; Fuller, R. C.; Lenz, R. W. *Macromol. Chem. Phys.* **1994**, *195*, 1405.
(12) (a) Hirt, T. D.; Neuenschwander, P.; Suter, U. W. *Macromol. Chem. Phys.* **1996**, *197*, 1609. (b) Hirt, T. D.; Neuenschwander, P.; Suter, U. W. *Macromol. Chem. Phys.* **1996**, *197*, 4253.
(13) Shi, F. Y.; Gross, R. A.; Rutherford, D. R. *Macromolecules* **1996**, *29*, 10.
(14) Madden, L. A.; Anderson, A. J.; Shah, D. T.; Asrar, J. *Int. J. Biol. Macromol.* **1999**, *25*, 43.
(15) Asrar, J.; Shah, D. T.; Tran, M. (Monsanto Co.) U.S. Patent 5,994,478, 1999.
(16) Shi, F. Y.; Ashby, R. D.; Gross, R. A. *Macromolecules* **1997**, *30*, 2521.
(17) Grassie, N.; Murray, E. J.; Holmes, P. A. *Polym. Degrad. Stab.* **1984**, *6*, 127.
(18) Grassie, N.; Murray, E. J.; Holmes, P. A. *Polym. Degrad. Stab.* **1984**, *6*, 95.
(19) Spyros, A.; Argyropoulos, D. S.; Marchessault, R. H. *Macromolecules* **1997**, *30*, 327.
(20) Shah, D. T.; Tran, M.; Berger, P. A.; Asrar; J.; Madden, L. A.; Anderson, A. J. Paper submitted for publication.

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