Biosynthesis and Characterization of Poly(3-hydroxy-4-pentenoic acid)

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ABSTRACT: *Burkholderia* sp. was grown on sucrose-containing mineral salts medium with phosphate limitation to induce poly(hydroxyalkanoate) (PHA) accumulation. Under these conditions the cultures accumulated 3-hydroxybutyric acid (3HB) and 3-hydroxy-4-pentenoic acid (3HPE) containing polyesters. Solvent fractionation of the purified polyester indicated the presence of two homopolymers, poly(3HB) and poly(3HPE), rather than a co-polyester with random monomer distribution as has been reported previously [Rodrigues, M. F. A.; da Silva, L. F.; Gomez, G. C.; Valentin, H. E.; Steinbüchel, A. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 880]. The simultaneous accumulation of two homopolyesters by *Burkholderia* sp. was confirmed by NMR spectroscopic analysis. Therefore, this is the first report on accumulation of a poly(3HPE) homopolyester and its accumulation from structurally unrelated carbon sources. Purified poly(3HPE) was cross-linked by UV radiation and subjected to epoxidation using 3-chloroperoxybenzoic acid. Introduction of epoxides into the 3HPE homopolyester was found to increase the glass transition temperature.

Introduction

Poly(hydroxyalkanoate)s (PHAs) are bacterial storage compounds that are accumulated when carbon and energy sources are available in excess, and growth is limited by the lack of an essential nutrient.[1] PHAs have useful thermoplastic properties and are currently evaluated as environmentally friendly plastics due to their production from renewable resources and biodegradability to carbon dioxide and water.[2] The most common PHA, poly(3-hydroxybutyrate) (poly(3HB)), is generally regarded to have poor material properties.[2,3] An exception is the recently discovered high molecular weight poly(3HB).[4] Research during the past 30 years has revealed that more than 100 different hydroxy fatty acids can be incorporated into PHA.[5] However, detailed analysis on the material properties of these different PHAs has been done in only a few cases. The random incorporation of 3-hydroxyvalerate or 4-hydroxybutyrate into a poly(3HB) backbone are among the best studied examples.[3,6] It is known, for example, that co-polyesters of 3HB and 3-hydroxyvalerate (3HV) are much less brittle than poly(3HB). In fact, poly(3HB-*co*-3HV), or Biopol, is the first PHA-based polyester produced through a fermentation process on a commercial scale.

Unfortunately, many of the unique PHAs with interesting material properties are produced only if the corresponding unique hydroxy fatty acids are included in the incubation medium as precursor substrates. Exceptions are, for example, 3HV[7] and medium-chain PHAs.[8] Recently, two new strains of *Burkholderia* sp. were isolated from soil samples and found to accumulate a polyester containing 3HB and 3-hydroxy-4-pentenoic acid (3HPE) when supplied with sucrose as the sole carbon and energy source.[9] It was reported that this polyester was a copolymer of 3HB and 3HPE.

Because of its unsaturation, the vinyl side chain of the 3HPE component is a potential target for polymer chemical modification. Depending on the modifying reagent, this could lead to improved material properties or a useful functionalized polymer. For example, poly(3HPE) might serve as a starting material to produce an emulsion-based biodegradable coating that could be cross-linked during processing. A polymer with low T_g, low degree of crystallinity, and a functionality which could be cross-linked would provide material with the potential for application in emulsion coating of paper and other substrates.

In this study it was discovered that *Burkholderia* sp. accumulates two distinct homopolyesters rather than a copolyester of 3HB and 3HPE. Such a formation of polymer blends has been found in wild type strains only once,[10] where polyesters accumulated by *Pseudomonas* strain were structurally very different, and the majority of precursors for these polymers appear to be derived from different pathways. It is interesting to note that, in contrast, the polyesters accumulated by *Burkholderia* sp. are structurally very similar. Most short-chain PHA synthases utilize C5−CoA esters in addition to C4−CoA esters as substrates, resulting in the formation of C4/C5 copolyesters. In fact, incorporation of 3HPE in a poly(3HB-*co*-3-hydroxyvalerate-*co*-3HPE) terpolyester by *Rhodospirillum rubrum* had been reported previously.[11] Therefore, the simultaneous accumulation of poly(3HB) and poly(3HPE) homopolymers by *Burkholderia* sp. appears to be unusual and raises interesting questions regarding the mechanisms involved in the formation of these polyesters.

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Experimental Section

Bacterial Strains and Growth Conditions. *Burkholderia* sp. DSMZ # 9243 was grown at 30 °C in nutrient broth (NB) or mineral salts medium[12] supplemented with an appropriate carbon source. Hoagland's trace element solution used in the original mineral salts medium described by Schlegel et al.[12] was replaced by trace element solution SL6.[13] For small scale fermentations, *Burkholderia* sp. DSMZ # 9243 was grown in a Braun Biostat B, 2 or 10 L fermentor, containing 4 g of NH_4SO_4, 2.2 g of $MgSO_4 \cdot 7H_2O$, 1.7 g of citric acid, and 10 mL of a trace element solution (containing 10 g of $FeSO_4 \cdot 7H_2O$, 2.25 g of $ZnSO_4 \cdot 7H_2O$, 0.5 g of $CuSO_4 \cdot 5H_2O$, 2 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of H_3BO_3, 0.1 g of $(NH_4)_6Mo_4O_{24}$, and 10 mL of HCl in 1 L) per liter. After autoclaving, this medium was supplemented with 3.5 g of KH_2PO_4 per liter and an appropriate carbon source. During fermentation, the pH was kept constant at pH 6.8 using ammonia or 20% sulfuric acid for pH adjustment. For aeration, 4 L of air was passed through the fermentor per minute, at an agitation rate of 730−1200 rpm (minimum DO = 20%). Fermentations were done at 34 °C and a 1 vol % of an overnight preculture was used as inoculum.

Quantitative and Qualitative Gas Chromatographic Analysis of PHA Polyesters. The polymer content and composition were analyzed by subjecting 3−15 mg of lyophilized cells to methanolysis in the presence of methanol−sulfuric acid (85:15, vol/vol) according to the method of Braunegg et al.[14] as modified by Brandl et al.[15] The methyl esters were separated according to Slater et al.[16]

Separation of the Poly(3HPE) from Polymer Blends. A sample of 3.84 g of PHA, obtained from a chloroform extraction of lyophilized *Burkholderia* sp. cells, was suspended in 50 mL of THF at 50 °C for approximately 45 min. Undissolved PHA was separated by filtration and dried in a vacuum oven at 60° C for 3 h. The THF insoluble portion, 3.46 g, was 97 mol % pure poly(3HB). The filtrate was evaporated to dryness to give 0.30 g of solid polymer containing 89 mol % poly(3HPE) and 11 mol % poly(3HB). When the separation was done without heating at room temperature, the filtrate contained 94 mol % poly(3HPE) and 6 mol % poly(3HB). Further purification with THF afforded 97 mol % pure poly(3HPE).

Epoxidation of Poly(3HPE). A solution of 1.5 equiv of 3-chloroperoxybenzoic acid, dissolved in 95 mL of chloroform, was added over a period of 35 min at 0 °C to a solution of 2.379 g of poly(3HPE) dissolved in 20 mL of chloroform. The reaction mixture was left stirring at room temperature overnight. Polymer, isolated in quantitative yield by precipitation in excess of methanol, was dried under vacuum overnight and used for thermal and spectroscopic analysis.

UV Cross-Linking of Poly(3HPE) Film. A 5 μm thick film of poly(3HPE) was cross-linked with UV by passing it through a UV processor equipped with a medium-pressure mercury vapor lamp. The exposure time was 5 min. No UV initiator was used.

Polymer Characterization. NMR spectra were obtained with SUN-based Varian UNITY spectrometers operating at 100.474 and 125.697 MHz ^{13}C frequencies and 399.601 and 500.617 MHz ^{1}H frequencies, respectively. The low-field (one-dimensional) spectra were acquired in a robot-operated spectrometer equipped with a four-nucleus probe maintained at 25 °C. Two-dimensional HMQC and HMBC[17] spectra were acquired at 500.617 MHz in an indirect detection probe maintained at 30 °C, under the following conditions: (1) ^{1}H (t_2) dimension: 98 ms acquisition time, 640 data points, zero-filled to 2048 data points, regular FT processing; (2) ^{13}C (t_1) dimension: 256 data points; linear prediction processing to 1024 data points.

Infrared spectra for all samples were recorded using a Nicolet Magna 550 coupled with a Spectra-Tech IR-Plan microscope. The spectra consist of 64 co-added scans collected at 4 cm^{-1} resolution. For sample preparation the polymer was rolled flat on a KBr chip prior to analysis. The spectra were normalized to the carbonyl band for comparison.

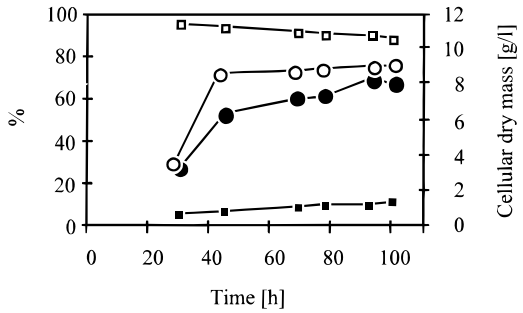

Figure 1. Poly(hydroxyalkanoate) accumulation by *Burkholderia* sp. Cells were grown in a 10 L fermentor using gluconate as a carbon and energy source and phosphate limitation for induction of poly(hydroxyalkanoate) accumulation. Abbreviations: ○, cellular dry mass; ●, % poly(hydroxyalkanoate) accumulation referred to the cellular dry mass; ■, accumulation of poly(3HPE) in mol %; □, accumulation of poly(3HB) in mol %.

Molecular weights were determined by GPC using a Waters 515 HPLC pump, connected to 10^3, 10^4, 10^5, and 10^6 Å Waters Ultrastyragel columns placed in series. The detection system consisted of a Waters 410 differential refractometer and a Viscotek T50 dual capillary viscometer connected in parallel. Chloroform was used as eluent at a flow rate of 1.0 mL/min. A sample volume of 100 μL with a polymer concentration of 2 mg/mL was typically used for analysis. 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. 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.

Thermal properties, glass transition temperatures, heat of fusion, and melting temperature were measured on Perkin-Elmer DSC-7. About 10 mg samples were encapsulated in standard aluminum DSC pans and heated from −50 to 125 °C. Poly(3HB) samples where heated to 180 °C. Heating and cooling rates were 20 °C/min. The glass transition temperature was taken as the midpoint of the total change in heat capacity. The peak temperature was reported as melting point. All calculations were performed on the first heating cycle. A pure indium metal was used to determine the temperature correction factor.

Results and Discussion

Characterization of PHA Isolated from *Burkholderia* sp. Recently, the formation of a poly(3-hydroxybutyrate-*co*-3-hydroxy-4-pentenoate) (poly(3HB-*co*-3HPE)) copolyester by *Burkholderia* sp. has been reported when this strain was grown on gluconate or sucrose as carbon source.[9] Highest 3HPE contents were obtained during growth on gluconate. The proportion of 3HPE increased continuously over time in these cultures. When these experiments were repeated in our hands (Figure 1), we confirmed the accumulation of 3HB and 3HPE containing polymers and a continued increase of the 3HPE fraction over time. However, the resulting polymer had a very broad molecular weight distribution with at least two distinct peaks (Figure 2). To ascertain whether the bimodality in molecular weight distribution has its origin in the polymer composition, fractionation of the chloroform extracted polymer was attempted. When this was done, it was found that the polymer produced by fermentation contained a mixture of THF-soluble and THF-insoluble fractions. The molecular weight of these polymers was found to be significantly different. The THF-soluble portion was of considerably lower molec-

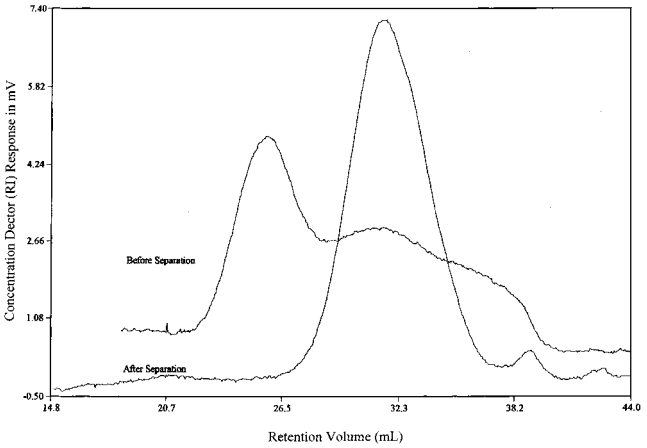

Figure 2. Molecular mass distribution of poly(hydroxyalkanoate)s obtained by chloroform extraction from *Burkholderia* sp.

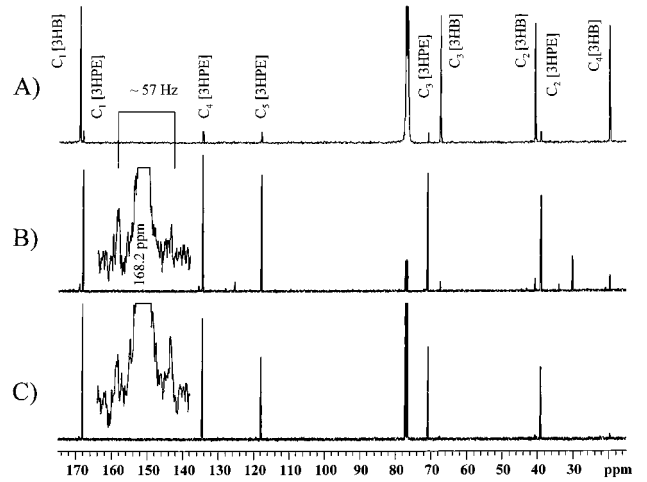

Figure 3. NMR spectroscopic analysis of polyesters extracted from *Burkholderia* sp. (A) represents a 100 MHz ^{13}C NMR spectrum of unpurified product of bacterial synthesis of poly(3HPE) (9 mol %). (B) ^{13}C NMR spectrum of 89 mol % pure poly(3HPE). (C) ^{13}C NMR spectrum of poly(3HPE) purified to more than 97 mol % by repeated THF extraction and ethanol precipitation.

ular weight (M_w = 94.9K) than the THF-insoluble portion (M_w = 1180K).

The ^{13}C NMR spectrum of bacterially produced polymer obtained through chloroform extraction consisted of two sets of peaks (Figure 3A): large-amplitude peaks with chemical shifts characteristic of poly(3HB) (91 ± 2 mol % of the total polymer based on ^1H NMR spectroscopy) and low-amplitude peaks attributed to poly(3HPE) (9 ± 2 mol %). The ^{13}C NMR spectrum of poly(3HB) is well-known and has been published,[18] and the spectrum of copolymerized 3HPE in poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-3-hydroxy-4-pentenoate) has been interpreted by Ulmer et al.[11,19] As shown in Table 1, the chemical shifts observed in this study are in excellent agreement with the published values. However, in contrast to the spectrum given in Ulmer et al.,[11] the ^{13}C NMR spectrum of 3HPE containing PHAs obtained from *Burkholderia* sp. lacked the dyad fine structure that is indicative of the compositional heterogeneity of a random copolymer. From this we concluded that the polymer was either a mixture of two separate polymers or a block copolymer of poly(3HB) and poly(3HPE).

Table 1. Chemical Shifts (ppm) of Poly(3HB) and Poly(3HPE)[a]

poly(3HPE)					poly(3HB)		
carbon no.	$^{13}C^b$	$^1H^b$	$^{13}C^c$	$^1H^c$	carbon no.	$^{13}C^b$	$^1H^b$
1	168.20		169		1	169.01	
2	39.09	2.57, 2.66	39	2.6	2	40.68	2.45, 2.57
3	70.99	5.58	71	5.6	3	67.60	5.25
4	134.51	5.78	134	5.8	4	19.66	1.25
5	117.96	5.17, 5.25	118	5.3			

[a] For assignment of carbon numbers see Figure 4. [b] This work. [c] Reference 11. Chemical shift data were taken out of the published spectra.

To resolve whether the polymer was a mixture or a block copolymer, a separation based on the two polymers' differential solubility in THF was performed. The ^{13}C NMR spectrum of the THF-insoluble fraction (dissolved) in $CDCl_3$ was almost completely attributable to poly(3HB) (not shown). The spectrum of the THF-soluble fraction, which was equally soluble in acetone, consisted of 89 ± 2 mol % poly(3HPE) and 11 ± 2 mol % poly(3HB) as determined by ^{13}C NMR spectroscopy (Figure 3B). By repeating the separation procedure, a purity of greater than 97 mol % poly(3HPE) was obtained (Figure 3C). Thus, the original isolated polymer was a mixture of two homopolymers and not a block copolymer.

As alluded to above, the carboxy region of the ^{13}C NMR spectrum is typically a sensitive indicator of compositional heterogeneity in PHAs. The peaks for pe*pe and b*b dyads in parts B and C of Figure 3 (168.23 and 169.16 ppm, respectively) are separated by 0.84 ppm, similar to that reported previously.[19] The weak peak at 168.59 ppm could be assigned to the dyad sum b*pe + pe*b. However, our spectroscopic data do not support such an assignment. Alternatively, this peak is interpreted as a ^{13}C satellite peak. We note that the 168.59 ppm peak, which is <1 mol % the intensity of the pe*pe peak, is matched by a symmetrically located peak upfield from the pe*pe peak, at 168.05 ppm. This is seen in the spectrum of both the sample containing 89 mol % and the sample containing >97 mol % 3HPE (Figure 3, B and C, respectively). The peak separation is 57 Hz, which is typical of *J* coupling constants between carboxy carbon and aliphatic carbon. In addition, if the pe*b and b*pe dyads were part of the 168.59 ppm peak, and coincidentally overlapped by the ^{13}C satellite peak, the relative carboxyl peak intensities would not match the intensities predicted by a single random distribution at the given comonomer composition. Relative peak intensities of the pe*b+b*pe dyads with respect to the pe*pe diad predicted for a random comonomer distribution would be 25% and 6% for the polymers containing 89% and 97% 3HPE units, respectively. The observed ratio of <1% is consistent with the calculated satellite ratio of 0.55%. This is convincing evidence in support of the resonance at 168.59 ppm being a ^{13}C satellite peak. As such, the levels of poly(3HB) seen in Figure 3B,C are confirmed as contaminants and not part of a random copolymer.

Heteronuclear correlation experiments involving one-bond (HMQC) and multiple-bond (HMBC) *J* coupling interactions were performed to confirm the peak assignments independently. The one-to-one correspondence between the chemical shifts of ^{13}C nuclei and directly attached protons is represented by the cross-peaks in Figure 5 (HMQC spectrum). Therefore, given the chemical shift of a ^{13}C nucleus on the horizontal

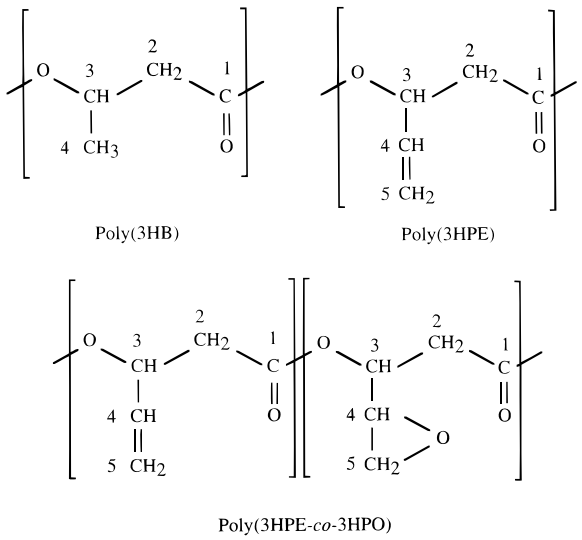

Figure 4. Structural formulas of poly(3HB), poly(3HPE), and poly(3HPE-*co*-3HPO).

axis, the chemical shift of the directly bonded proton can be read off the vertical axis at the height of the cross-peak. The diagram confirms all of the ^{13}C chemical shift assignments for 3HPE and 3HB presented in Table 1. The multiple bond correlation diagram (HMBC spectrum) in Figure 6 shows cross-peaks representing one-to-one correspondence between ^{13}C nuclei and protons that are two or three bonds removed. For poly(3HPE), all but two (H_4 to C_5 and H_4 to C_2) of the expected correlations were detected, thereby establishing the structure beyond a doubt.

These results lead us to the conclusion that *Burkholderia* sp. accumulates two structurally different homopolyesters rather than a block co-polyester or a copolyester as assumed previously.[9] The biochemical mechanisms that enable the accumulation of these two structurally related polyesters as separate homopolyesters in an overlapping time frame (Figure 1) are unknown. Simultaneous accumulation of blends containing poly(3HB) and medium-chain PHAs[20] or a poly(3HB) homopolyester and a poly(3-hydroxybutyrate-*co*-3-hydroxyalkanoate) copolymer[10] requires the presence of distinct PHA synthases.[10,20] The accumulated polymer types were determined by the substrate specificities of the PHA synthases. Southern hybridization experiments designed to probe for PHA synthase genes in total genomic DNA of *Burkholderia* sp. suggested the presence of more than one synthase gene.[21] However, our efforts to clone these two PHA synthase genes resulted in the cloning of a genomic region encoding a *R. eutropha*-like PHB operon only.[21]

Cross-Linking Purified Poly(3HPE). In an effort to produce cross-linked polymer, a film of poly(3HPE) on KBr was exposed to UV irradiation. The FTIR of the poly(3HPE) film obtained before and after UV exposure is shown in Figure 7. The =CH stretch at 3095 cm^{-1} and the C=C stretch at 1647 cm^{-1} present in the poly(3HPE) film before the UV exposure diminish considerably as the film is exposed to UV, indicating that the double bond in poly(3HPE) has participated in a cross-linking reaction. No optimization of UV exposure was attempted since cross-linked polymer was found to be relatively brittle compared to the linear poly(3HPE). This is probably due to a high degree of cross-linking as apparent from the near total disappearance of the double bond in the FTIR spectrum.

Epoxidized Poly(3HPE). The double bonds in poly(3HPE) provide an interesting opportunity as a reaction site for polymer modification. PHAs containing vinyl pendent groups have previously been epoxidized with complete conversion using perbenzoic acid as an oxidizing agent.[22] Epoxidation of poly(3HPE) was carried out under similar conditions. The ^{13}C NMR spectra of more than 97 mol % pure bacterially produced poly(3HPE) (major impurity poly(3HB), Figure 3C) and of the peroxidation product are shown in Figure 8. The chemical shifts of the major peaks are listed in Table 2 (refer to Figure 4 for the structure with assigned carbons). The oxidation has only been partial, as indicated by the magnitude of the olefinic peaks at 135 and 118 ppm. The peaks at 51 and 45 ppm are unique to the oxidation product, and their chemical shifts are characteristic for epoxides;[23] they therefore confirm oxidation of the double bond of poly(3HPE) to form poly(3-hydroxy-4-epoxyvaleric acid) (poly(3HPO)).

Integration of the peaks assigned to 3HPE and 3HPO (Table 2, Figure 8) indicates a composition of 55 mol % 3HPE and 45 mol % 3HPO for the oxidized polymer. Since the peroxide oxidation is not stereospecific, the asymmetric center of the epoxide moiety, C_4(O), has both *R* and *S* symmetry, while the C_3 of 3HPO is still stereospecifically pure *R* or *S*. Thus, the formation of the various diastereomers through this reaction partially explains the complexity of the spectrum (refer to the expanded portions of the spectrum in Figure 8). However, the patterns are more complex than commonly observed in PHA copolymers and cannot be explained by compositional heterogeneity of a poly(3HPE-*co*-3HPO) copolymer alone. As a result of the nonstereospecific nature of the peroxidation reaction, the ^{13}C spectrum fine structure reflects both stereo- and regioheterogeneity. Thus, nine environments (instead of four in the absence of stereoheterogeneity) account for the ^{13}C spectrum of the carboxy dyads—O^R*O^R, O^R *O^S, O^R *E; O^S * O^R, O^S * O^S, O^S *E; E* O^R, E* O^S, E*E—and of the E-centered triads of C_3(E): O^R E* O^R, O^R E* O^S, O^S E* O^R, O^S E* O^S, EE* O^R, EE* O^S, O^R E*E, O^S E*E, EE*E (O^R, O^S, and E are abbreviations for the comonomers 3HPO^R, 3HPO^S, and 3HPE, respectively). Not all positions are resolved in the experimental ^{13}C spectrum (Figure 8). The carboxy signals at 168 ppm show five distinct peaks, and two peaks are suggested by shoulders. The extended spectrum of C_3(E) (71 ppm) resolves eight peaks which are grouped in three pairs around a dominant center peak. This is in qualitative agreement with the expectation for a random polymer, where O^R E* O^S, O^S E* O^R; EE* O^R, O^R E*E; and EE* O^S, O^S E*E have equal intensity. The C_3 peaks that are due to 3HPE and 3HPO monomer units in the epoxidized copolymer are clearly separated by their chemical shifts (Figure 8, inset). The well-resolved multiplet at 71 ppm is attributed to the 3HPE monomer unit, and the two less defined resonances at 70.3 and 69.4 ppm are attributed to 3HPO^R and 3HPO^S monomer units. The epoxy peaks (C_4 and C_5, at 51 and 45 ppm) are also clearly split into optical isomers. The ratio of the two symmetries (*R, S*) is 2/3, as determined by integration of the peaks at 51.93, 51.20, 45.46, and 44.79 ppm and by the peaks at 70.3 and 69.4 ppm.

In summary, the peroxide oxidation of more than 97 mol % pure poly(3HPE) produced a copolymer of 3HPE and 3HPO, consisting of 55 mol % 3HPE and 45 mol % 3HPO. The peroxide oxidation of the double bond of

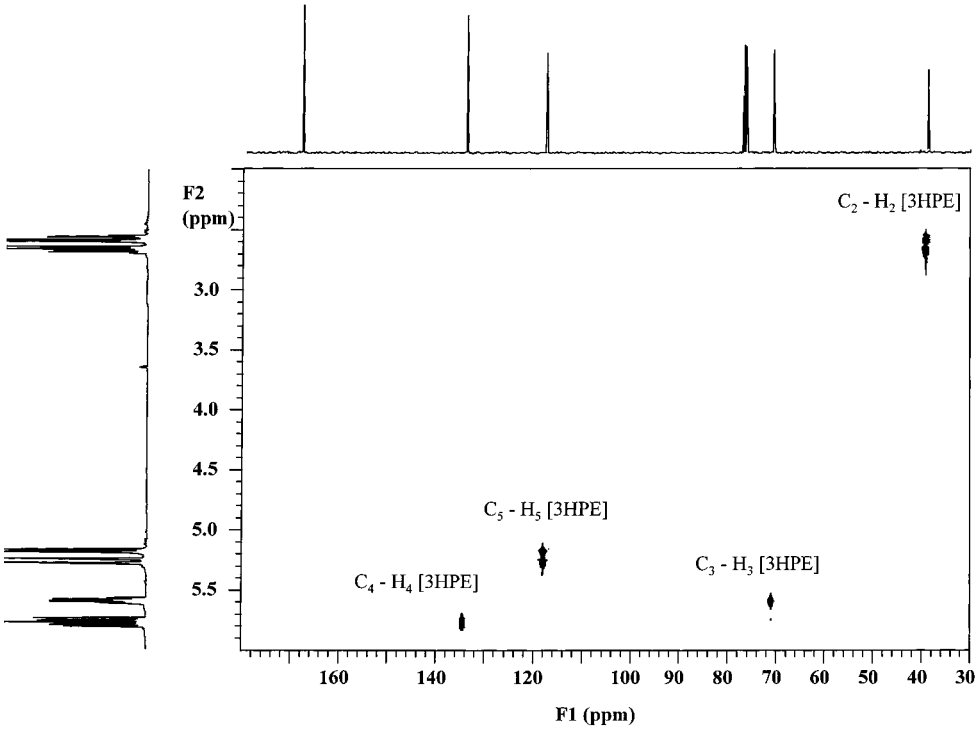

Figure 5. The 125 MHz one-bond $^{1}H-^{13}C$ correlation spectrum (HMQC) of 89 mol % poly(3HPE) sample.

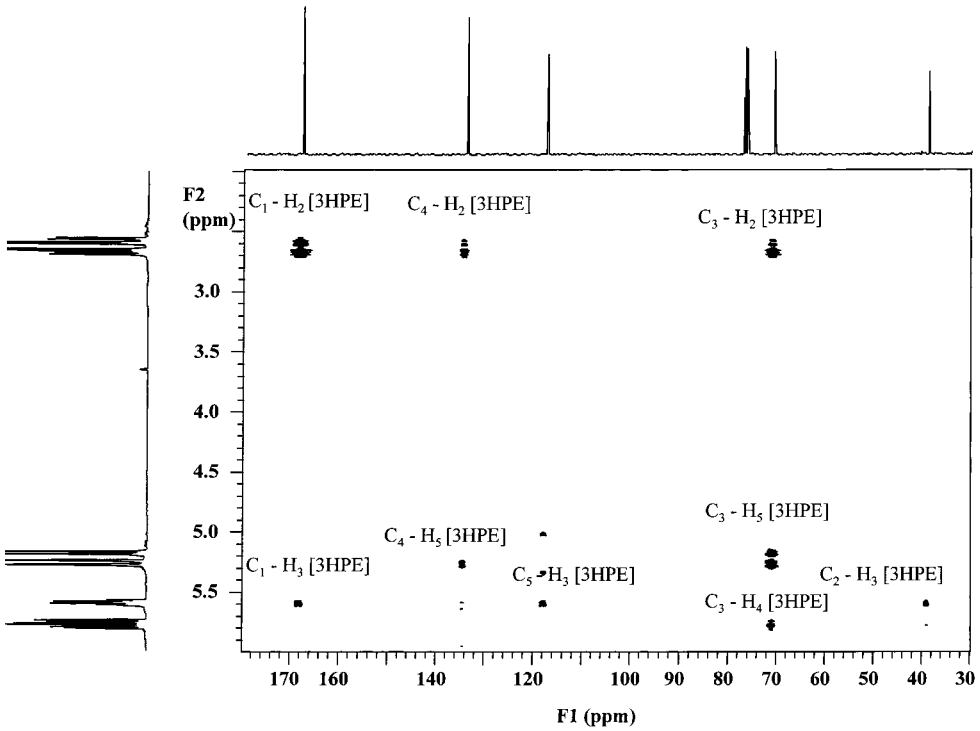

Figure 6. The 125 MHz multiple-bond $^{1}H-^{13}C$ correlation spectrum (HMBC) of 89 mol % poly(3HPE) sample.

poly(3HPE) was not stereospecific and resulted in a 2/3 (*R*/*S*) ratio of stereoisomers. Both stereo- and regioregularity are reflected in the fine structure of the ^{13}C NMR spectrum.

Thermal Properties. The thermal behavior of the poly(3HPE) and epoxidized poly(3HPE) (poly(3HPO)) was studied by DSC and compared with poly(3HB) and poly(3HB-*co*-3HV). The fermentation product, which is a blend of poly(3HB) and about 10 mol % poly(3HPE), has melting and crystallization temperatures very similar to those of poly(3HB) (Table 3). As the polymer is purified by removing the THF-soluble poly(3HPE) fractions, a slight increase in the melting temperature, heat of fusion, and heat of crystallization is observed. The poly(3HPE) homopolyester, compared to poly(3HB) and poly(3HV), is found to have very low crystallinity and a lower melting temperature. This polymer is also very slow to crystallize. During the cooling process crystallization was not observed. Altering the side group in PHAs from the ethyl group of poly(3HV) to a vinyl group as found in poly(3HPE) did not have significant effect on the glass transition temperature. Epoxidation of the vinyl groups in poly(3HPE), however, considerably increased the glass transition temperature of the poly-

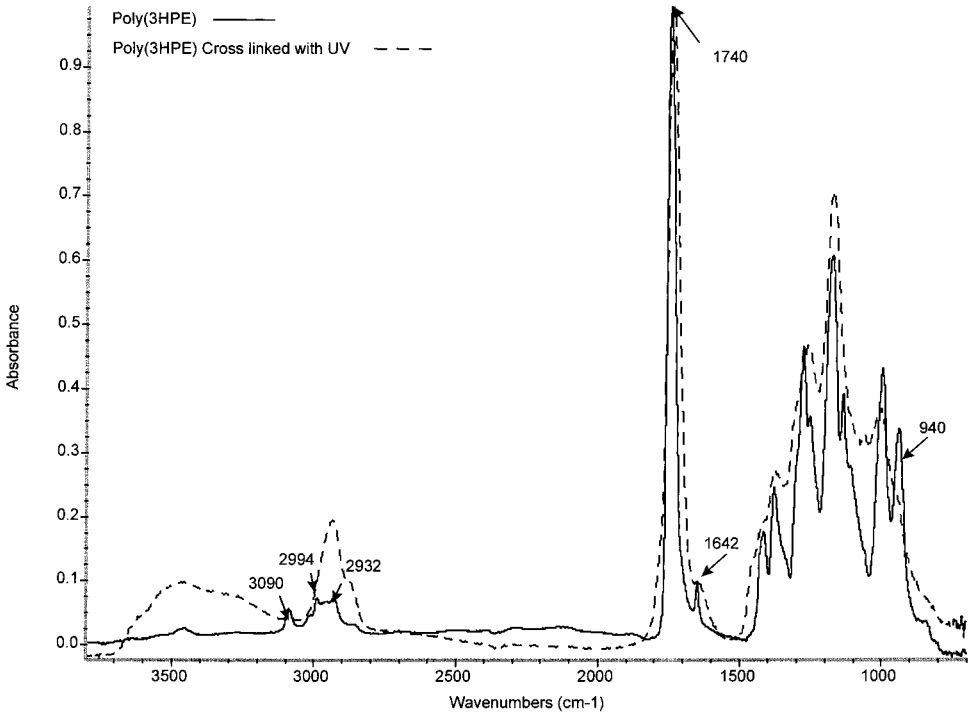

Figure 7. FTIR of poly(3HPE) film prior and post UV cross-linking.

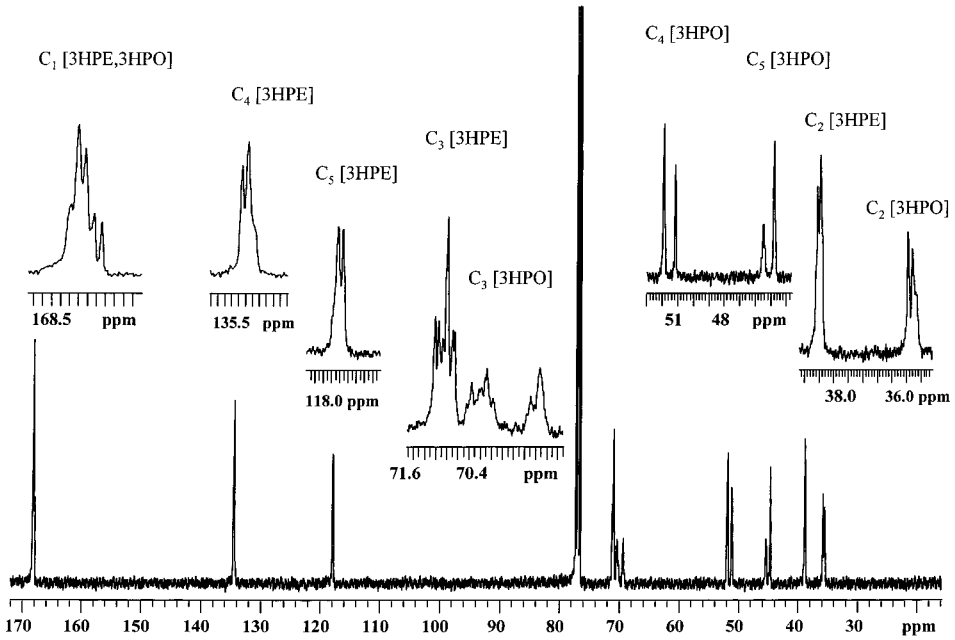

Figure 8. The 100 MHz ^{13}C NMR spectrum of partially epoxidized poly(3HPE) obtained from the expoxiation of poly(3HPE).

mer by 45%. These results are very similar to the effect observed on the epoxidation of natural rubber and that of poly(3-hydroxyoctanoate-*co*-3-hydroxy-10-decenoate) where the increase in T_g is attributed to the increased intramolecular interactions due to the high polarity of epoxide groups.[22]

Conclusion

Burkholderia sp. DSMZ # 9243 is able to accumulate polyesters containing 3HB or 3HPE from structurally unrelated carbon sources.[9] Molecular weight analysis of the polyester isolated from various fermentation experiments revealed a bimodal molecular weight distribution. Using solvent fractionation, we were able to separate two polymer species, whereby the final THF-soluble polymer fraction contained more than 97 mol % poly(3HPE). NMR spectroscopic analysis of polymer blends and solvent fractionated polyesters revealed that *Burkholderia* sp. in fact accumulates two distinct homopolyesters—a poly(3HB) homopolyester and a poly(3HPE) homopolyester—simultaneously.

The presence of unsaturated R pendent groups in poly(3HPE) is found to considerably lower the degree of crystallinity and the melting point compared to the fully saturated analogue poly(3HV) without significantly effecting the T_g. Presence of unsaturation in the polymer also provides an opportunity for cross-linking and further polymer modification. Epoxidation of poly(3HPE) was found to increase the glass transition temperature.

Table 2. ^{13}C Chemical Shifts of Poly(3HPE) and of Epoxidized Poly(3HPE)[a]

carbon no.	stereospecificity	poly(3HPE)	poly(3HPO)
C1 (3HPE), C1 (3HPO)		168.22	168.31 168.23 168.14 168.05
C4 (3HPE)		134.56	134.56 134.47
C5 (3HPE)		117.989	118.07
C3 (3HPE)		71.02	71.02
C3 (3HPO)	(*R* or *S*)		70.29
	(*S* or *R*)		69.35
C4 (3HPO)	(*R* or *S*)		51.93
	(*S* or *R*)		51.20
C5 (3HPO)	(*S* or *R*)		45.46
	(*R* or *S*)		44.79
C2 (3HPE)		39.15	38.98
C2 (3HPO)			35.97

[a] The data were obtained by ^{13}C NMR spectroscopic analysis of greater than 97 mol % pure poly(3HPE) and the epoxidation product of this polymer, poly(3HPE-*co*-3HPO).

Table 3. Effect of PHA Side-Chain Structure on Their Thermal Properties[a]

polymer	T_g, °C	T_m, °C (H, J/g)	T_c, °C (H, J/g)
fermentation product, poly(3HB)/poly(3HPE) blend	nd	167.3 (90.4)	110 (−78.9)
THF insoluble fraction, poly(3HB)	nd	170.5 (94.2)	97.1 (−81.4)
THF soluble fraction, poly(3HPE)	−10.8	62.7 (14.5)	nd
poly(3HV)	−13.7	110.1 (63.7)	71.2 (59.5)
poly(3HPE-*co*-3HPO)	+6.9	nd	nd

[a] Abbreviations: nd, not determined; THF, tetrahydrofuran.

The biosynthetic pathway(s) for accumulation of poly(3HB) and poly(3HPE) homopolyesters in *Burkholderia* sp. remains an interesting open question. The fact that two homopolymers of distinctly different molecular weights are produced and that fermentation experiments with 4-pentenoic acid as a supplement had no effect on 3HPE accumulation (data not shown) may suggest that the two polymerization processes for poly(3HB) and poly(3HPE) formation are not biochemically linked.

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