Stereoregularity in Synthetic β-Hydroxybutyrate and β-Hydroxyvalerate Homopolyesters

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ABSTRACT: High molecular weight poly(β-hydroxybutyrate) (PHB) and poly(β-hydroxyvalerate) (PHV) were synthesized from racemic β-butyrolactone and β-valerolactone, respectively, with an oligomeric alumoxane catalyst obtained by reaction of $AlEt_3$ with H_2O. High-field ^{13}C NMR spectroscopy resolved stereosequences in PHB and PHV at the diad level and showed that the $AlEt_3/H_2O$ catalyst produced partially stereoregular polymer. ^1H NMR at 250 MHz showed peak splittings corresponding to partial resolution of triad sequences. Adjusting the $AlEt_3/H_2O$ mole ratio allowed maximization of the degree of stereoregularity. Extraction of whole polymer with ethanol or acetone separated the polyesters into a soluble, low molecular weight atactic fraction and an insoluble, high molecular weight isotactic fraction. The insoluble fractions were shown by NMR to have a high level of isotacticity (85-87% isotactic diads). These fractions also showed substantial crystallinity. Fractionated synthetic PHB corresponds closely to optically active bacterial PHB in melting point, enthalpy of fusion, and X-ray crystal structure. The X-ray crystal lattice of the synthetic PHV prepared in this study is in good agreement with that of a bacterial PHV previously obtained from sewage sludge as a mixture with PHB.

Introduction

Poly(β-(*R*)-hydroxybutyrate) (PHB) is a naturally occurring polyester synthesized by a wide variety of bacteria.[1] It functions as both a source of energy and carbon supply for many bacteria and is relatively abundant in the environment in, for example, estuarine microflora,[2] blue-green algae,[3] microbially treated sewage,[4] and soil bacterial colonies.[5] PHB is optically active, and in the crystalline state it adopts a compact right-handed helix with a twofold screw axis and a fiber repeat of 5.96 Å.[6-8] It is found in the form of granules in the cytoplasmic fluid and normally comprises 1-30% of the dry weight of the bacterial cells; however, controlled fermentation encourages overproduction of polymer to as much as 80% of the dry cell weight.[9,10] This technology has been developed into a large-scale fermentation process by ICI, and PHB is currently being marketed as the "first thermoplastic from biotechnology".[11]

Several authors have described the synthesis of analogues of bacterial PHB by nonbiochemical methods. Polycondensation of β-hydroxybutyric acid does not lead to high polymer, since the acid readily dehydrates to crotonic acid.[12] PHB and similar polyesters have been synthesized, however, by ring-opening polymerization of substituted β-lactones.[13-17] Early syntheses of PHB from racemic β-butyrolactone using various acidic and basic,[14] as well as organometallic,[16] catalysts led to low molecular weight polymers with properties very different from those of bacterial PHB. In 1962 Cherdron et al. described the polymerization of β-propiolactone using as catalyst the reaction product of triethylaluminum and water.[15] Agostini et al. first reported the synthesis of partially stereoregular PHB, employing the same $AlEt_3/H_2O$ system. From racemic (±)-β-butyrolactone (β-BL) they obtained partially crystalline but optically inactive PHB,[18] and from *R*-(+)-β-BL of 73% optical purity they claimed to have synthesized an optically active analogue of the natural polymer.[19] Tani et al. reported the synthesis of PHB from racemic β-BL having higher molecular weight and achieved an increased crystallinity by means of solvent extraction.[20-22] Particularly interesting was the observation by both groups that the X-ray diffraction patterns of synthetic PHB derived from racemic or partially optically active monomer were identical with that of bacterial PHB.[8,18,19]

The nonbiochemical synthesis of poly(β-hydroxyvalerate) (PHV) from racemic β-valerolactone (β-VL), carried out under the same conditions as the synthesis of PHB, has been reported by Tani et al.[21,22] By X-ray diffraction studies these authors showed that crystalline PHV adopts a compact twofold helical conformation with a fiber repeat of 5.56 Å, similar to the helical conformation of PHB.[23] In single crystals grown from a sample derived from sewage sludge, two distinct populations of crystals corresponding to PHB and PHV were identified.[4,24-26] Unit-cell dimensions obtained by electron diffraction on selected single crystals from this natural sample showed excellent agreement with those of synthetic PHV.

The polymerization of racemic β-BL and β-VL by an achiral catalyst to a product having the same helical crystal structure as an optically active biopolymer is most unusual. No such stereoregulation is exhibited by $AlEt_3$ in the absence of water or by the Inoue catalyst, (tetraphenylporphinato)aluminum chloride.[27-31] The $ZnEt_2/H_2O$ system polymerizes β-BL at a higher rate than the $AlEt_3/H_2O$ system but gives PHB having very low crystallinity. Attempts to prepare optically active PHB by using the stereoelective $ZnEt_2$/*R*-(−)-3,3-dimethyl-1,2-butanediol catalyst system gave polyesters of only moderate enantiomeric enrichments.[32]

The polymerization of β-lactones using the alumoxane catalyst (eq 1 and 2), formed by reacting $AlEt_3$ with H_2O, can be optimized to yield crystalline polymer. Tani and co-workers observed a maximum yield of whole polymer and of a crystalline, acetone-insoluble fraction at a catalyst composition corresponding to $[H_2O]/[AlEt_3] = 1.0$.[22] The authors were unable to determine the stereoregularity of their polymers using 100-MHz ^1H NMR spectroscopy and instead relied on crystallinity information obtained from

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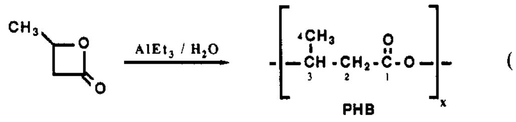
IR spectroscopy and X-ray diffraction measurements. Their conclusion was that the crystalline fraction of synthetic PHB consists of equal numbers of right- and left-handed helices formed from stereoregular chains.[8,23]



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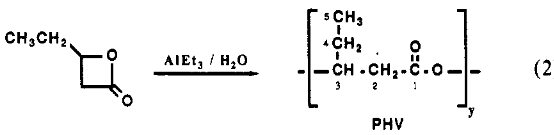

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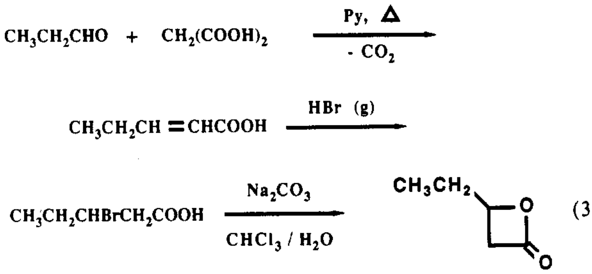
In 1986 several groups recorded the ^1H and ^{13}C NMR spectra of bacterial PHB and related copolyesters under higher resolution conditions than Tani et al. in 1978.[33-36] These results suggested that NMR spectroscopy could be a useful tool for studying tacticity and sequence effects in the analogous copolyesters synthesized by a nonbiochemical route. In this paper we describe the use of ^1H and ^{13}C NMR to study stereoregulation in the synthesis of PHB and PHV from racemic β-lactones using the $AlEt_3/H_2O$ catalyst system. The following paper in this issue explores the further complication of comonomer sequence distribution in synthetic HB/HV copolyesters.

Experimental Section

Materials. All chemicals, except β-pentenoic acid, β-bromopentanoic acid, and β-valerolactone, were obtained commercially. β-Butyrolactone (β-BL) and triethylaluminum (toluene solution) were obtained from Aldrich Chemical Co. For use as a polymerization solvent, chloroform was dried by refluxing over P_2O_5, distilled under vacuum and stored under N_2 over 4-Å molecular sieves. The bacterial PHB sample used in this work was produced by ICI, Agricultural Division, Billingham, U.K.

Racemic β-valerolactone (β-VL) was prepared by reacting propionaldehyde with malonic acid to form β-pentenoic acid via the Knoevenagel reaction,[37] followed by β-bromination and lactonization as shown in eq 3.



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β-Pentenoic Acid.[37-40] A flask was equipped with a mechanical stirrer and a dry ice condenser fitted with a $CaCl_2$ drying tube. To 206 g (2.6 mol) of dry pyridine, cooled to 0 °C, 204 g (2 mol) of dry malonic acid and then 146 g (2.5 mol) of dry propionaldehyde were added with constant stirring. The ice–water bath was removed, and the mixture was left stirring at room temperature. Additional 0.5-mol portions of malonic acid were added on the second and third days of the reaction. After 6 days, 400 mL of 50% sulfuric acid was added with stirring to the ice-cooled mixture. Cooling of the resulting mixture caused most of the β-pentenoic acid to separate as a clear oil. The remaining aqueous layer was extracted 6 times with 75–100-mL portions of diethyl ether, and the combined organic fractions were dried over anhydrous $MgSO_4$. After filtration, ether was removed by rotary evaporation and the crude product fractionally distilled through a Vigreux column at reduced pressure to give β-pentenoic acid: 82% yield, >99% purity by ^1H NMR; ^1H NMR ($CDCl_3$, 80 MHz) δ 12.19 (s, 1 H), 7.18 (m, 1 H), 5.82 (d, 1 H), 2.27 (m, 2 H), 1.08 (t, 3 H).

(±)-β-Bromopentanoic Acid.[41-45] β-Pentenoic acid (30 g) was saturated with dry HBr gas at room temperature and HBr bubbled periodically through the stirred solution. The conversion to β-bromopentanoic acid was followed by ^1H NMR and exceeded 99% after 7 days. The crude product was fractionally distilled twice under high vacuum to give pure β-bromopentanoic acid: 97% yield; mp 59 °C [lit. mp 58.5–59 °C];[43] ^1H NMR ($CDCl_3$, 250 MHz) δ 11.39 (s, 1 H), 4.29 (m, 1 H), 2.97 (d, 2 H), 1.88 (m, 2 H), 1.07 (t, 3 H). When the reaction was repeated with 280 g of β-pentenoic acid, the conversion was only 80% after 17 days. Removal of β-pentenoic acid was achieved by recrystallization of the product from hexane/chloroform, rather than by distillation.

(±)-β-Valerolactone. To a stirred solution of 70 g (0.39 mol) of β-bromopentanoic acid in 600 mL of $CHCl_3$, a solution of 32 g (0.30 mol) of Na_2CO_3 in 200 mL of water was added slowly. The mixture was stirred vigorously for 6 h at 40 °C. The water phase was extracted with $CHCl_3$, the combined organic phases were dried over $MgSO_4$, and the solvent was removed under vacuum. The crude β-VL was dried by stirring overnight over CaH_2 at room temperature and was fractionally distilled twice under high vacuum and stored under N_2 over 4-Å molecular sieves: 70% yield; >99% purity by ^1H NMR; n^{20}_D 1.4191 [lit. n^{20}_D 1.4190];[21] IR (neat) 1822 cm^{-1}; ^1H NMR ($CDCl_3$, 250 MHz) δ 4.48 (m, 1 H), 3.29 (quartet of d, 2 H), 1.86 (m, 2 H), 1.02 (t, 3 H). A similar purification and drying procedure was used for β-BL.

Polymerization. Dry monomer (5.00 mL; $\rho = 1.0471$ g/mL for β-BL; $\rho = 1.0106$ g/mL for β-VL) was added to a high-vacuum ampule, and a dry N_2 feed line was inserted, allowing a steady gas flow. Two milliliters of a 1.9 M $AlEt_3$ solution in toluene was added to the ampule at −78 °C, followed by dry $CHCl_3$, where appropriate. The N_2 purge was then removed, and an accurately measured amount of water (0–130.0 μL) was added by microsyringe before the ampule was mounted on a vacuum line. Polymerization mixtures were degassed by three freeze/pump/thaw cycles to a residual pressure of 5×10^{-5} mbar. Polymerizations were carried out at 60 °C for 7 days. Depending on the amount of $CHCl_3$ solvent, the viscosity generally increased considerably with time. Yellow products resulted from reactions with catalyst compositions $[H_2O]/[AlEt_3] \leq 1.0$; the intensity of the color decreased as the $H_2O/AlEt_3$ ratio was increased. Samples were diluted with $CHCl_3$ to a final polymer concentration of 1–2 wt % and, if necessary, were worked up by dissolving the polymerization mixture at 60 °C. The solutions were added dropwise to 500 mL of diethyl ether/petroleum ether (75/25 for PHB; 25/75 for PHV) containing a few volume percent of water, a procedure which usually yielded a flocculant precipitate of polymer. This material is referred to as whole polymer.

Fractionation of Polymer. The precipitated polymer samples were extracted with acetone (PHB) or ethanol (PHV) for 5–10 h in a Soxhlet apparatus and then stirred in acetylacetone[22] (10 mL/g of polymer) for 2 days in order to remove the alumoxane catalyst. The polymers were precipitated with ethanol (5 mL/mL of acetylacetone), centrifuged at 10 000 rpm, washed twice with diethyl ether, recentrifuged, and air-dried. Destruction of the catalyst in this fashion removed the yellow color and greatly increased the solubility of the resulting polymer. Acetone was found to be a more effective solvent than ethanol for removing atactic polymer from PHB.

Polymer Characterization. ^1H and ^{13}C NMR spectra of 1–2 wt % solutions of polymer in $CDCl_3$ were recorded on Bruker WM 250 or AM 250 instruments using DISNMR software. ^1H NMR spectra were recorded at ambient temperature with a 2500-Hz spectral width, 45° (2-μs) pulse, 2-s recycle delay, and 32–128 transients. A 16K FID was acquired and zero-filled to 32K before Fourier transformation. Proton-decoupled 62.9-MHz ^{13}C NMR spectra were obtained at ambient temperature with a 12 500-Hz spectral width, 45° (12.5-μs) pulse, 2-s recycle delay, 32K data points, and 10 000–30 000 transients. Under these conditions the relative intensities of peaks within the multiplet of a given carbon resonance are quantitatively reliable.[34,36] Aluminum analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, by inductively coupled plasma (ICP) spectrometry, a technique with an Al detection limit of ~10 ppm. DSC thermograms were recorded with a Du Pont 9900 thermal analyzer at heating rates of 20, 10, and 5 °C/min. The temperature and ΔH values were calibrated with tin and indium standards. Heating rates slower than 5 °C/min were not used to avoid possible thermal degradation of the polyester sample. Melting points were determined

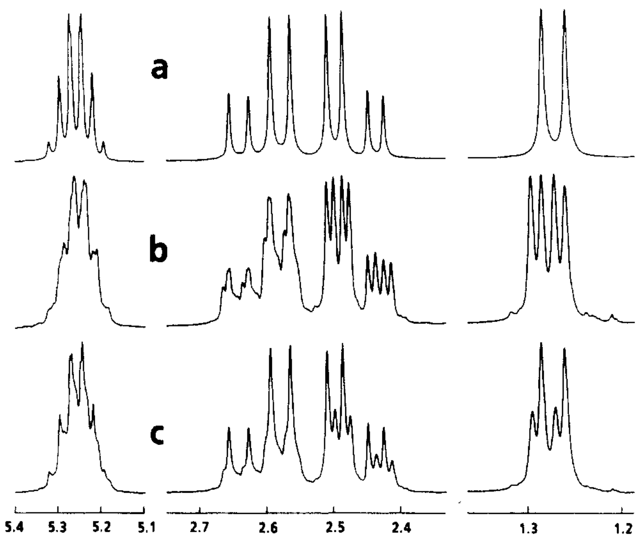

Figure 1. 250-MHz ^1H NMR spectra of (a) bacterial poly(β-(R)-hydroxybutyrate) (PHB), (b) atactic PHB prepared with $AlEt_3$ catalyst, and (c) an ~50/50 physical mixture of a and b.

by extrapolation to zero heating rate. In the case of multiple endotherms the maximum of the higher temperature endotherm was taken as the true melting point. Diffractometer traces were collected by using a Philips Model PW 1050/80 X-ray powder diffractometer equipped with a nickel-filtered Cu Kα source (λ = 0.1542 nm), graphite monochromator, and pulse-height analyzer. X-ray powder patterns were collected by using a Warhus flat film camera on a Philips Model 1730 X-ray generator. Kodak DEF-5 X-ray film and Cu Kα radiation were used. Viscosities of filtered polymer solutions in $CHCl_3$ were measured at 30.0 ± 0.05 °C in an Ubbelohde viscometer. Typically 4–5 dilutions over a concentration range of 0.025–0.3 wt % polymer were made. The viscosity-average molecular weight, M_v, was calculated from the intrinsic viscosity, $[\eta]$, which was determined by extrapolating the reduced specific viscosity, $(\eta/\eta_0 - 1)/c$, and the inherent viscosity, $\ln(\eta/\eta_0)/c$, to zero concentration. GPC analyses were performed for 1 wt % polymer solutions in $CHCl_3$ (1.0 mL/min) at 30 °C by Dr. J. Moore of the Ontario Research Foundation with a Waters GPC II instrument equipped with 10^5-, 10^4-, and 10^3-Å Waters μ-styragel columns and a differential refractometer detector.

Results and Discussion

Synthesis of Stereoregular PHB. At sufficiently high field both ^1H and ^{13}C NMR provide information on tacticity effects in synthetic PHB and PHV. The 250-MHz ^1H NMR spectrum of bacterial poly(β-(R)-hydroxybutyrate) in $CDCl_3$ solution is shown in Figure 1a. The methine proton attached to the asymmetric carbon is a multiplet at 5.25 ppm, the methylene multiplet at 2.53 ppm consists of eight peaks due to the conformational inequivalence of the two hydrogens, and the CH_3 signal is a simple doublet at 1.27 ppm.

If racemic β-butyrolactone (β-BL) is polymerized with triethylaluminum catalyst in the absence of water, a nonstereoregular or atactic polymer results.[21] Figure 1b shows clearly that the ^1H NMR spectrum of such a sample is more complex than that of the optically active bacterial polymer (Figure 1a). In particular, where bacterial PHB displays a CH_3 doublet at 1.27 ppm, the CH_3 resonance of atactic PHB (Figure 1b) shows two overlapping doublets. This doubling is also seen in the methylene resonance but is incompletely resolved in the more complex methine signal. Figure 1c shows the ^1H NMR spectrum of an approximately 50/50 mixture of bacterial (isotactic) PHB and synthetic (atactic) PHB. On the basis of this addition experiment, the observed splittings are attributed to tacticity effects. The peak splittings correspond to diad or partially resolved triad sequences.

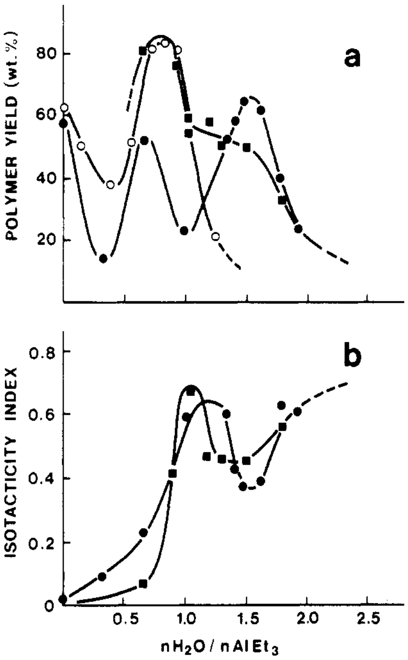

Figure 2. Optimization of polymerization of racemic β-butyrolactone: (a) total yield of whole polymer versus catalyst composition and (b) isotacticity index versus catalyst composition. ○, data from ref 21, no solvent; ■, this work, no solvent; ●, this work, 62 vol % $CHCl_3$ in polymerization mixture.

Because the CH_3 group is directly attached to an asymmetric center which is equidistant from the two adjacent asymmetric centers, its ^1H NMR spectrum should, in principle, be analyzed in terms of configurational triads. For polymers with true asymmetric centers and a sense of chain direction, such as PHB, there are four NMR-distinguishable triads: *RRR* + *SSS*, *RRS* + *SSR*, *RSS* + *SRR*, and *RSR* + *SRS*. In the notation of Sepulchre,[46] these four triads are designated *ii*, *is*, *si*, and *ss*, respectively, where *i* and *s* denote isotactic (*RR*,*SS*) and syndiotactic (*RS*,*SR*) diads.[47] On the basis of this analysis, four separate ^1H NMR doublets are expected for the CH_3 group of synthetic PHB. However, at 250 MHz only two doublets are resolved; attempts to resolve three or all four doublets by varying the solvent and temperature were unsuccessful. At least one of the contributions to the low-frequency CH_3 doublet (Figure 1b) is due to *ii* sequences since this resonance has the same chemical shift as that of bacterial PHB (Figure 1c). In the absence of more highly resolved spectra, the assignment of the remaining triads is ambiguous. The high-frequency doublet could, for example, be assigned to any one of *ss*, *si*, *is*, *ss* + *si*, *ss* + *is*, *si* + *is*, or *ss* + *si* + *is* triads. If it is assigned to *ss* and one of the two heterotactic sequences, *si* or *is*, the analysis is reduced to the diad level since $(i) = (ii) + {}^1/_2[(is) + (si)]$ and $(s) = (ss) + {}^1/_2[(is) + (si)]$. Without corroborating evidence (e.g., ^{13}C NMR spectra), an unambiguous assignment of the ^1H spectra, except for *ii* triads, is impossible.

In experiments designed to optimize the overall polymer yield and yield of stereoregular polymer, racemic β-BL was polymerized under various conditions. The $H_2O/AlEt_3$ ratio was the most important variable in this respect. Spectra similar to that of Figure 1c were obtained for whole polymer samples polymerized with different catalyst compositions. The CH_3 group spectra differed only in the relative intensities of the high- and low-frequency doublets. Figure 2a shows that there are three ratios which corre-

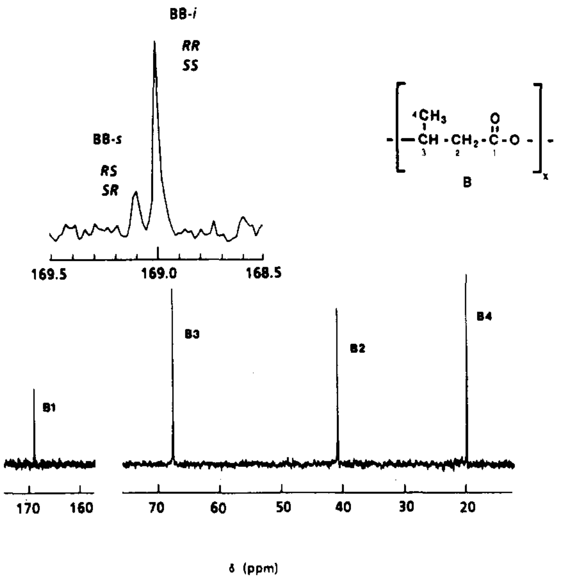

Figure 3. 63-MHz ^{13}C NMR spectrum of PHB polymerized under optimum stereoregulating conditions.

spond to relative maxima in percent conversion, suggesting that at least three different organoaluminum species are responsible for polymerizing racemic β-BL to substantial yields. Partial agreement was obtained with the previous work of Tani et al.;[21] however, these authors reported few results above a 1:1 molar ratio of $H_2O/AlEt_3$, where stereoregulation by the catalyst is highest. In the present work the stereoregulating capacity of the catalyst was determined by ^1H NMR and is expressed in terms of an isotacticity index, defined as

$$\text{isotacticity index} = (H_l - H_h)/H_l$$

where H_l and H_h are the peak heights of the low- and high-frequency CH_3 group doublets, respectively. This index is not an absolute measure of isotacticity due to the incomplete triad resolution and ambiguity in peak assignments. Figure 2b shows a well-defined maximum in isotacticity index at $[H_2O]/[AlEt_3] = 1.0$, indicating that the stereoregulating capacity of the catalyst is highest at this composition. Another maximum is observed around $[H_2O]/[AlEt_3] = 2.0$. However, the 1:1 ratio is the optimum catalyst composition, since under these conditions the total yield of polymer is much higher. The addition of $CHCl_3$ to the polymerization mixture, originally intended to facilitate workup, reduced the total yield of stereoregular polymer. This is shown in Figure 2 for polymerization of β-BL (62 vol % of $CHCl_3$), where maxima in polymer yield correspond to minima in the level of isotacticity. Polymerization in 82 and 90 vol % of $CHCl_3$ further reduced the total yield of polymer.

^{13}C NMR spectroscopy can also be used to determine the degree of stereoregularity of synthetic PHB. Figure 3 shows the 63-MHz ^{13}C NMR spectrum of a whole sample of PHB polymerized under optimum conditions. Previously Araki and co-workers observed only a slight shoulder on the carbonyl resonance in the 25-MHz ^{13}C spectrum of synthetic PHB.[48] At 63 MHz, however, the carbonyl resonance clearly shows two peaks due to tacticity effects. Under the same conditions, the carbonyl ^{13}C resonance of bacterial poly(β-(R)-hydroxybutyrate) shows only one signal at 169.05 ppm.

Since the C=O group is equidistant from adjacent asymmetric centers, the ^{13}C NMR spectra can be analyzed in terms of *i* (*RR*,*SS*) and *s* (*RS*,*SR*) configurational diads. The peak at 169.05 ppm, corresponding to that of isotactic bacterial PHB, is assigned to *i* diads and the additional peak at 169.16 ppm to *s* diads (Figure 3). Equal intensity of *i* and *s* peaks implies an absence of stereoregularity (atacticity) since there are, on average, as many *RR*/*SS* as *RS*/*SR* sequences per polymer chain. Conversely, a low relative intensity of *s* to *i* diads implies a high degree of stereoregularity. Figure 4 shows that catalyst optimization leads to marked changes in both the ^1H and ^{13}C NMR spectra of synthetic PHB. The relative intensities of the two methyl resonances in the ^1H NMR spectra for various catalyst compositions (Figure 4a–c) parallel the relative intensities of the *i* and *s* carbonyl peaks in the corresponding ^{13}C NMR spectra (Figure 4e–g). Again, equal intensity of the high- and low-frequency doublets in the ^1H NMR spectrum indicates atacticity, since in the ^{13}C NMR spectrum of the same sample the *s* and *i* C=O diad peaks are of similar intensity. A low relative intensity of the high-frequency doublet corresponds to a high degree of stereoregularity; in the ^{13}C NMR spectrum a low relative intensity of *s* to *i* C=O peaks is observed. The close similarity between the relative peak heights of the high- and low-frequency methyl group ^1H doublets and the relative intensities of the *s* and *i* diad carbonyl ^{13}C peaks strongly suggests that the high-frequency CH_3 doublet should be assigned to *ss* and one of the heterotactic *si* or *is* triads, as discussed above. A rigorous quantitative comparison of the ^1H and ^{13}C NMR intensities was not attempted, however, because of the severe overlap of the CH_3 doublets and the relatively low signal-to-noise of the C=O ^{13}C spectra.

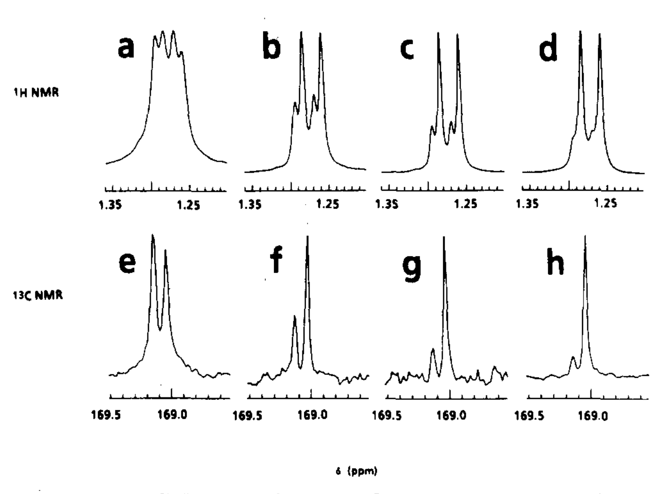

Figure 4. (a–d) Expanded plots of CH_3 resonances in 250-MHz ^1H NMR spectra of synthetic PHB samples. (e–h) Expanded plots of C=O resonances in 63-MHz ^{13}C NMR spectra of synthetic PHB samples. $[H_2O]/[AlEt_3]$ = 0:1 (a, e); 2:1 (b, f); 1:1 (c, g); 1:1 after extraction with acetone for 14 h, catalyst removal, and precipitation (d, h).

Aside from catalyst optimization, additional improvements in the degree of stereoregularity of synthetic PHB were obtained by acetone extraction of the more soluble, atactic polymer. The effect of extraction of a whole sample of PHB on the relative intensity of the *s* diad ^{13}C carbonyl resonance is shown in parts g and h of Figure 4. The relative intensity of the *s* diad resonance of the soluble fraction was similar to that of atactic PHB (Figure 4e). Comparison of Figure 4c,d with Figure 4g,h shows that the ^1H NMR chemical shifts are less sensitive to the degree of stereoregularity than the ^{13}C NMR chemical shifts.

Removal of Catalyst Residue from Synthetic PHB. The alumoxane catalyst is tightly bound to the polymer but can be removed by an appropriate chelating agent. Table I summarizes the analytical results for aluminum in synthetic PHB by ICP spectroscopy. The analyses of three whole samples of PHB indicate that the catalyst

Table I
Removal of Catalyst Residue from Synthetic PHB

	polymerization conditions			aluminum content, wt %	
polymer sample	$[H_2O]/[AlEt_3]$	vol % $CHCl_3$[a]	sample description	calcd[b]	obsd[c]
1	1.5	62	whole sample	4.1	4.3
1	1.5	62	EtOH-soluble fraction		5.2
1	1.5	62	EtOH-insoluble fraction		5.0
2	1.5	0	whole sample	3.7	3.5
2	1.5	0	triturated with 0.1 M HCl		1.8
3	1.0	0	whole sample	3.6	3.0
3	1.0	0	acetylacetone treated		0.33

[a] Volume percent of $CHCl_3$ in β-BL/$CHCl_3$ polymerization mixture. [b] Based on the amount of $AlEt_3$ added relative to the total yield of polymer. [c] Measured by ICP spectrometry.

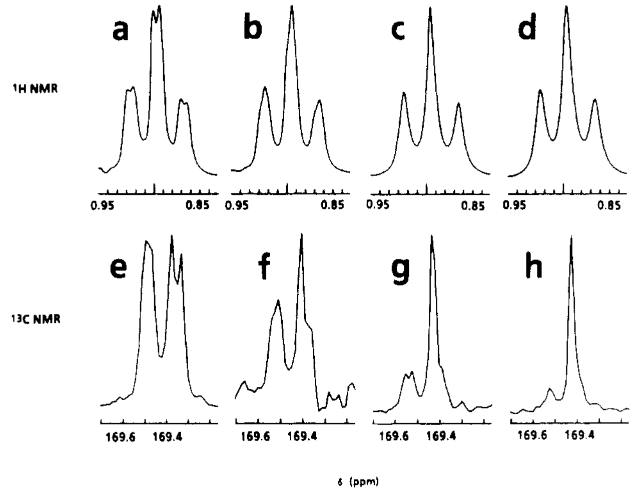

Figure 5. (a-d) Expanded plots of CH_3 resonances in 250-MHz ^1H NMR spectra of synthetic PHV samples. (e-h) Expanded plots of C=O resonances in 63-MHz ^{13}C NMR spectra of synthetic PHV samples. $[H_2O]/[AlEt_3]$ = 0:1 (a, e); 1:1 (b, f); 1:1, insoluble fraction after extraction with EtOH for 14 h (c, g); 1:1, insoluble fraction after extraction with EtOH for 14 h, catalyst removal, and precipitation (d, h).

remains with the polymer after precipitation. This is seen in Table I from the close agreement between the calculated aluminum content, based on the amount of $AlEt_3$ added relative to the total yield of polymer, and the observed content. Thorough extraction of the polymer with EtOH did not remove aluminum from the insoluble fraction (sample 1, Table I). Catalyst removal by trituration with hydrochloric acid, as performed by Agostini and coworkers,[18,19] reduced the aluminum content of the polymer by ~50% (sample 2). However, this is not a recommended procedure since the acid may hydrolyze the polyester and thus reduce the molecular weight. The result for sample 3 shows that treatment with a chelating agent such as acetylacetone[22] is most effective in removing the residual catalyst.

Synthesis of Stereoregular PHV. Racemic β-VL, polymerized under the same conditions as β-BL followed by EtOH fractionation, also yielded highly stereoregular polymer. The CH_3 resonance in the ^1H NMR spectrum of an atactic synthetic PHV sample (Figure 5a) is split into two overlapping triplets. As for PHB, the splitting is attributed to partial resolution of *ii*, *is*, *si*, and *ss* triads. Figure 5b illustrates that ^1H NMR spectroscopy is much less sensitive to tacticity effects for PHV than for PHB. This is also apparent in parts c and d of Figure 5, which show ^1H NMR spectra of the insoluble fractions from successive extractions of the whole polymer sample whose spectrum is given in Figure 5b.

Table II
Isotacticity of Synthetic PHB and PHV

polymer	catalyst composition $[H_2O]/[AlEt_3]$	sample description	isotactic diads,[a] %
PHB	1.5	whole sample	62
PHB	1.0	whole sample	78
PHB	1.0	acetone-insoluble fraction	85
PHV	1.0	whole sample	43
PHV	1.0	EtOH-insoluble fraction	87

[a] Determined from carbonyl peak areas in 63-MHz ^{13}C NMR spectra.

Although ^1H NMR is less useful as a probe of tacticity for PHV, ^{13}C NMR is just as effective for PHV as for PHB (Figure 5e–h). Extraction of atactic material from a whole sample of PHV is demonstrated by the decreased relative intensity of the *s* diad resonance for the ^{13}C NMR spectra shown in Figure 5f–h; the carbonyl resonances of synthetic PHV are more complex than those of synthetic PHB, due to longer range tacticity effects (i.e., partial resolution of tetrads). A comparison of the ^{13}C NMR spectra of whole samples of PHB and PHV (Figures 4g and 5f, respectively) suggests that, for a given $AlEt_3/H_2O$ ratio, the catalyst is more stereoselective in the polymerization of racemic β-BL than in the polymerization of racemic β-VL.

Isotacticity of Fractionated PHB and PHV. The level of isotacticity of whole samples of synthetic PHB and PHV can be increased significantly by solvent extraction of the more soluble atactic material. Here, the degree of isotacticity was quantified from the simpler and better resolved ^{13}C NMR spectra, rather than by ^1H NMR. The isotacticity was calculated from the peak area corresponding to *i* diads divided by the total area due to *i* + *s* diads. Isotactic diad contents of 62% and 78% for the first and second PHB samples in Table II confirm that the catalyst composition has an effect on the stereoregularity of the resulting polymer. The isotacticity of the latter sample could be increased to 85% by extraction with acetone. The whole sample of PHV has an isotactic diad content of only 43%; this confirms that the alumoxane catalyst is more stereoregulating for β-BL than for β-VL. Extraction of this whole sample of PHV, however, increased the isotactic diad content to 87%.

Such high isotacticities indicate that fractionated synthetic PHB and PHV contain long ~*RRR*~ and ~*SSS*~ sequences. Tani and co-workers[8,23] have previously suggested that the alumoxane catalyst produces racemic polyesters consisting of equal numbers of purely *R* and *S* chains. In this model, the small fraction of syndiotactic diads observed by NMR would be due to occasional defects in an otherwise isotactic chain. Such defects would also give rise to *ss* triad sequences. An alternative model for synthetic PHB and PHV is a stereoblock structure with alternating long ~*RRR*~ and ~*SSS*~ sequences in the same polymer chain. Each block junction, the result of steric propagation errors, corresponds to *is* and *si* configurational sequences; *ss* triads do not occur. The independent isotactic chain and stereoblock models are thus potentially differentiable by NMR spectroscopy.[49] If *ss* triad resonances are observed, the isotactic chain model (with periodic defects) is indicated. Conversely, the absence of *ss* triad resonances supports the stereoblock model. In the present case, where the ^1H CH_3 triads are incompletely resolved and only diads are observed in the

Table III
Molecular Weight Analysis for PHB and PHV

polymer sample	$[\eta]$,[a] dL/g	$M_v \times 10^{-3}$,[b,c] g/mol	$[\eta']$,[d] dL/g	$M_n \times 10^{-3}$,[e] g/mol	M_w/M_n[e]
PHB					
bacterial	2.17	293,[b] 275[c]	3.30	161	2.1
synthetic, acetone soluble	0.080	4.0,[b] 4.0[c]			
synthetic, acetone insoluble	1.46	177,[b] 166[c]	1.40	21.4	5.6
PHV					
synthetic, EtOH soluble	0.058	2.4[c]			
synthetic, EtOH insoluble	1.78	190[c]	1.99	52.1	3.4

[a] Determined viscometrically. [b] Calculated from $[\eta]$; $K = 1.18 \times 10^{-4}$ and $a = 0.78$ from ref 51. [c] Calculated from $[\eta]$; K estimated by the method of ref 52, assuming $a = 0.78$. [d] Calculated using M_i from GPC data and K and a from ref 51. [e] Determined by GPC, calibrated to polystyrene standard and corrected to K and a of PHB.

C=O ^{13}C NMR spectra, an unambiguous conclusion is not possible. However, the stereoblock model is consistent with what is known of the mechanism of ring-opening polymerization of β-lactones, in which stereoselectivity is the result of the directing effect of the alumoxane coordinated chain end (i.e., the last inserted monomer unit). Moreover, it is difficult to envision a plausable mechanism by which an achiral homogeneous catalyst can produce a racemic mixture of highly isotactic R and S polymer chains from a racemic monomer.

Molecular Weight of Synthetic PHB and PHV. Table III lists the intrinsic viscosities of fractionated samples of synthetic PHB and PHV following extraction with acetone and ethanol, respectively. Viscosity-average molecular weights, M_v, calculated for PHB by using literature values for the constants K and a in the Mark–Houwink–Sakurada equation,[50,51] are also listed in Table III together with data for a sample of bacterial PHB. In the case of PHV these coefficients were not available in the literature, and K was calculated to be 1.36×10^{-4} dL/g by the procedure of Van Krevelen,[52] assuming the same value of $a = 0.78$ as for PHB.[51] The validity of this approach was shown by a similar estimation of K for PHB ($K_{obsd} = 1.18 \times 10^{-4}$ dL/g;[51] $K_{est} = 1.24 \times 10^{-4}$ dL/g), which yielded a value of M_v very close to the experimental value. The results in Table III show that the alumoxane catalyst yields low molecular weight atactic fractions and high molecular weight isotactic fractions for both PHB and PHV. The viscosity-average molecular weight of the isotactic fraction of PHB is comparable to that of bacterial PHB. The GPC results further confirm the relatively high molecular weight of these synthetic polyesters; the molecular weight distributions are somewhat broader than for bacterial PHB. Barham et al.[53] have reported molecular weights for a series of bacterial PHB samples obtained by GPC at 30 °C in $CHCl_3$. For four different samples, M_n ranged from 20 000 to 250 000 and M_w ranged from 35 000 to 468 000; $M_w/M_n = 1.75–2.7$.[53] Since both synthetic and bacterial PHB are of high molecular weight, direct comparison of such physical properties as melting point, enthalpy of fusion, and degree of crystallinity can be made.

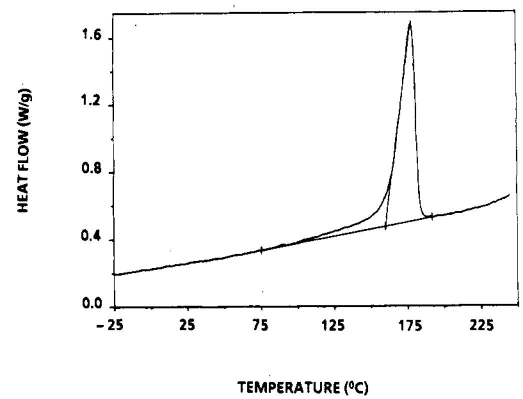

Figure 6. DSC thermogram of bacterial PHB (T_m = 179 °C and ΔH_m = 25 cal/g). Heating rate 10 °C/min.

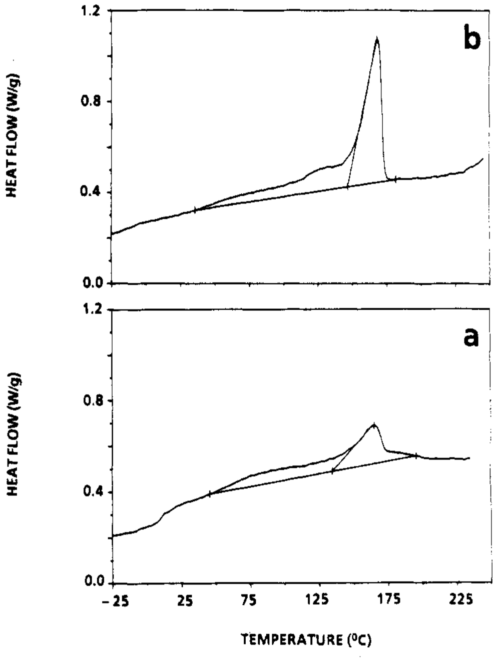

Figure 7. DSC thermogram of synthetic PHB: (a) whole polymer sample (T_m = 165 °C and ΔH_m = 11.9 cal/g) and (b) acetone-insoluble fraction (T_m = 170 °C and ΔH_m = 22.4 cal/g). Heating rate 10 °C/min.

Crystallinity of Synthetic PHB and PHV. Figure 6 shows the DSC thermogram of a sample of bacterial PHB having a degree of crystallinity of 64 ± 5% as determined previously by wide-angle X-ray diffraction (WAXD).[36] This sample exhibits a sharp melting endotherm at 179 °C, and the area under the curve corresponds to an enthalpy of fusion, ΔH_m, of 25 cal/g. The absence of a marked glass transition is consistent with the high degree of crystallinity of this sample. Figure 7a is the DSC thermogram of the whole sample of synthetic PHB whose NMR spectra are shown in Figure 4c,g. Clearly, this sample is less stereoregular than the bacterial reference material, as demonstrated by the broad melting range, the lower melting point (T_m = 165 °C), and the lower enthalpy of fusion (ΔH_m = 12 cal/g). A glass transition, visible near 5 °C, is further evidence for the low degree of crystallinity of this sample. Removal of the atactic material by solvent extraction left a 28 wt % fraction with a narrower melting range, higher melting point (T_m = 170 °C), and larger enthalpy of fusion (ΔH_m = 23 cal/g), as shown in Figure 7b (for the corresponding ^{13}C NMR spectrum, see Figure 4h). Although the ΔH_m values of this sample and bacterial PHB are very similar, it should be noted that the synthetic sample melts over a wider temperature range, a conse-

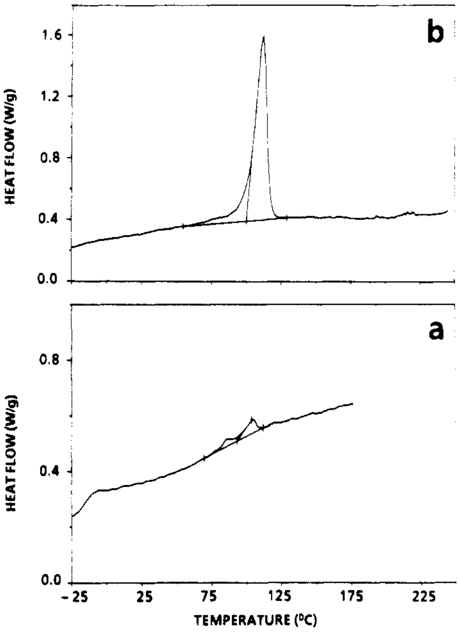

Figure 8. DSC thermogram of synthetic PHV: (a) whole polymer sample (T_m = 103 °C and ΔH_m = 1 cal/g) and (b) EtOH-insoluble fraction (T_m = 112 °C and ΔH_m = 20.0 cal/g). Heating rate 10 °C/min.

quence of the presence of ~15% of syndiotactic diads. Tani et al. reported a melting point of 167–169 °C for synthetic PHB.[8] The WAXD patterns observed for our synthetic sample were identical with those of bacterial PHB.[54] Similar observations have also been made by Agostini et al.[18,19] and Tani et al.[8] for their samples of synthetic PHB.

Figure 8 shows the effect of solvent extraction on the DSC trace of synthetic PHV. The whole polymer sample in Figure 8a displays a prominent glass transition near -20 °C, a very low enthalpy of fusion (ΔH_m = 1 cal/g), and a low melting point (T_m = 103 °C). However, careful fractionation with ethanol yielded a 5 wt % insoluble fraction with a high level of isotacticity (87% isotactic diads), as shown in Figure 5h. The corresponding DSC scan is shown in Figure 8b; it displays no glass transition. Instead, it shows a sharp melting endotherm at 112 °C, with a much higher ΔH_m of 20 cal/g. Tani and co-workers have reported melting points of 102–110 °C for synthetic PHV.[22,23] The WAXD pattern observed for the EtOH insoluble fraction of synthetic PHV was identical with that of bacterial PHV obtained from a physical mixture of PHB and PHV.[25,26] Additional WAXD data and a comparison with copolymers of HB and HV are presented in the following paper.[54]

Conclusions

We have shown by high-field ^{1}H and ^{13}C NMR spectroscopy that the $AlEt_3/H_2O$ catalyst system developed by Agostini, Lando, and Shelton[18] for the polymerization of substituted β-lactones has significant stereoregulating capability. The catalyst incorporates *R* and *S* monomers stereoselectively to give a small fraction of highly isotactic chains. By taking advantage of the difference in solubility between atactic and isotactic polymer,[21,22] we have isolated highly stereoregular fractions of PHB and PHV. These fractions were shown to have high molecular weights and were crystalline. In contrast, the soluble fractions were of low molecular weight and noncrystalline. Since stereoregular high molecular weight synthetic PHB resembles optically active bacterial PHB so closely in such physical properties as X-ray crystal structure,[54] melting point, and enthalpy of fusion, it is likely that right- and left-handed helical segments of the polymer chains are aligned in separate crystalline domains.

Acknowledgment. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada. Steven Bloembergen thanks NSERC, Xerox Corp., and the University of Waterloo for scholarship support.

Registry No. β-VL, 53580-31-7; β-VL (homopolymer), 53580-32-8; β-VL (SRU), 67291-18-3; β-BL (homopolymer), 36521-53-6; β-BL (SRU), 26744-04-7; H_2O, 7732-18-5; $AlEt_3$, 97-93-8; β-pentenoic acid, 626-98-2; malonic acid, 141-82-2; propionaldehyde, 123-38-6; β-bromopentanoic acid, 118375-92-1.

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Isodimorphism in Synthetic Poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate): Stereoregular Copolyesters from Racemic β-Lactones

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Received September 15, 1988

ABSTRACT: Analogues of bacterial poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate) have been synthesized by ring-opening polymerization of mixtures of racemic β-butyrolactone and β-valerolactone with a stereoselective alumoxane catalyst. Atactic fractions were removed by extraction with ethanol or acetone, leaving behind high molecular weight fractions with enhanced stereoregularity and sharp melting points. Whereas bacterial β-hydroxybutyrate/β-hydroxyvalerate polyesters are commercially available for only a limited range of comonomer compositions, the full composition range was obtained synthetically. Approximately the same random comonomer sequence distribution was found for the synthetic materials as for the natural copolyesters. Copolymer compositions were determined by 250-MHz ^{1}H NMR spectroscopy, which is also sensitive to tacticity effects. Tacticity and comonomer sequence effects were investigated in further detail by 63-MHz ^{13}C NMR spectroscopy. As was previously observed for the bacterial copolyesters, the fractionated synthetic analogues were highly crystalline and displayed isodimorphism, a phenomenon in which the crystal lattice of either homopolymer can accommodate the repeating unit of the other monomer.

Introduction

Poly(β-(*R*)-hydroxybutyrate) (PHB) and poly(β-(*R*)-hydroxybutyrate-*co*-β-(*R*)-hydroxyvalerate) (P(HB-*co*-HV)) are the first commercial thermoplastics from a bacterial source. Whereas PHB is a relatively abundant bacterial polyester,[1-6] poly(β-(*R*)-hydroxyvalerate) (PHV) has only been observed in a mixture with other polyalkanoates isolated from sewage sludge.[6-9] The Agricultural Division of ICI has developed a fermentation process for the production of these polyesters from petrochemical as well as nonpetrochemical feedstocks.[10,11] Acetate derived from these feedstocks is the building block for PHB homopolymer. The addition of propionate to the growing culture results in P(HB-*co*-HV).[12] These optically active copolyesters are manufactured in a large-scale fermenter by ICI from *Alcaligenes eutrophus* and are now available in kilogram quantities under the trade name Biopol.[11,12] Although copolymers containing up to 47 mol % β-hydroxyvalerate (HV) have been isolated,[13,14] the upper limit of HV content obtainable in the high-yield fermentation process is 20–30 mol %.[15] The apparent upper limit of HV incorporation is related not to enzyme selectivity but rather to the propionate level in the feed, since this is toxic to the bacteria in the culture. As a consequence, low copolyester yields are obtained for high propionate feeds. Nevertheless, copolyesters grown from *A. eutrophus* have a random comonomer distribution at all available compositions.[16,17]

The bacterial P(HB-*co*-HV) system has several unusual physical properties which have encouraged us to explore the entire composition range of synthetic analogues from 0–100% HV. Despite being a random copolymer, bacterial P(HB-*co*-HV) is crystalline at all accessible compositions. For bacterial samples containing 0–47 mol % HV we have measured degrees of crystallinity by wide-angle X-ray diffraction of 61–74% ± 5%.[16] Depending upon composition, individual copolyesters crystallize in either the PHB unit cell[18-21] or the PHV unit cell.[8,9,22] A crystal lattice transition occurs at ~30 mol % HV.[16] The lattice transition manifests itself at a minimum or pseudoeutectic point in plots of melting point or enthalpy of fusion against copolymer composition[16] and is probably related to packing energy considerations. This phenomenon of mutually

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0024-9297/89/2222-1663$01.50/0
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