Stereochemistry of the Ring-Opening Polymerization of (*S*)-β-Butyrolactone

Yan Zhang, Richard A. Gross,† and Robert W. Lenz*

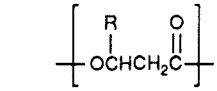
Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

Received July 31, 1989; Revised Manuscript Received January 17, 1990

ABSTRACT: The stereochemical course of the ring-opening polymerization of β-butyrolactone, BL, catalyzed by triethylaluminum/water and diethylzinc/water catalysts was studied using (*S*)-BL as a stereochemical probe. The (*S*)-BL monomer, which was prepared in five steps from optically pure poly[(*R*)-β-hydroxybutyrate], P[(*R*)-HB], produced by bacteria, had an optical purity in excess of 97%. The stereochemical configuration and isomeric purity of the repeating units in the polymers obtained were determined both from their specific optical rotation and by degradation of the polymers to their component methyl β-hydroxybutyrate units. The isomeric purity of these methyl esters was determined from their 200-MHz ^{1}H NMR spectra in the presence of a chiral europium shift reagent, and the diad stereochemical sequence distributions of the polymers were determined by 75.4-MHz ^{13}C NMR spectroscopy on appropriate samples. From these investigations it was concluded that (1) the mode of ring opening for an $AlEt_3/H_2O$ (1/1) catalyst prepared in situ involved primarily cleavage of the bond between the β-carbon and oxygen of the lactone (alkyl cleavage) with inversion of configuration equal to or greater than 93%, (2) the mode of ring opening using the preformed ethylaluminoxane (EAO) catalyst proceeded primarily by bond breaking between the carbonyl carbon and oxygen of the lactone (acyl cleavage) with 85% retention of configuration, and (3) with the $ZnEt_2/H_2O$ (1/0.6) catalyst prepared in situ ring opening occurred by acyl cleavage with retention of configuration in excess of 97%. The stereoisomeric purities of different molecular weight fractions of the polymers prepared with the EAO catalyst were found to differ dramatically, suggesting that the EAO catalyst prepared in the present study contained more than one type of active site, each of which caused the polymerization of BL to proceed by different ring-opening pathways.

Introduction

Poly[(*R*)-β-hydroxyalkanoates], PHAs, are synthesized by a variety of bacteria[1-4] and function as intracellular carbon and energy storage materials.[1] In most cases, the PHAs produced by microorganisms, as shown below, consist mainly of β-hydroxybutyrate, HB, and β-hydroxyvalerate, HV, repeating units,[2-4] but smaller quantities of other repeating units with pendant groups as long as *n*-nonyl have also been reported.[2]

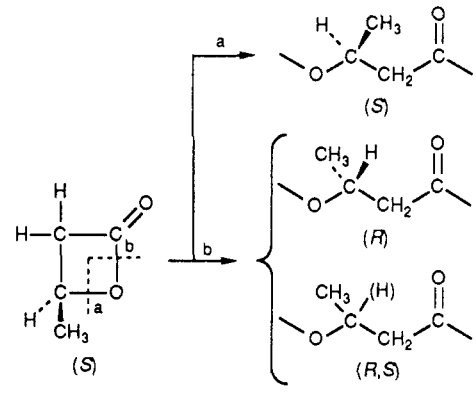


R = $-(CH_2)_xCH_3$ (x = 0-9)

The synthesis of structural and stereochemical isomers of such PHAs can also be carried out by the ring-opening polymerization reactions of appropriate β-monosubstituted β-propiolactones.[5] These synthetic analogues are useful for understanding the physical properties and biological activity of PHAs from bacterial origin, which, in many cases, are produced only as copolymers in a limited range of compositions.[6-11] That is, the polymerization of β-monosubstituted β-propiolactones can be used to synthesize homopolymers and copolymers that are not currently available from the biosynthetic route, but the ability to control the stereochemistry of the ring-opening polymerization of these monomers is essential in order to obtain synthetic analogues of the bacterial PHAs, all of which are believed to contain the (*R*) chiral center at the β-position.

In this report a synthetic route is described for the preparation of (*S*)-butyrolactone, (*S*)-BL, in high optical purity from a bacterial polyester that contains only (*R*)-HB repeating units. (*S*)-BL was used as a stereochemical probe to determine the mode of ring opening for three different coordination catalysts that have been used to prepare PHB from BL. In this type of polymerization reaction ring opening of a lactone may proceed by bond breaking either between the carbonyl carbon and oxygen atom of the β-lactone ring (acyl cleavage) with retention of configuration as shown in path a (Scheme I) below or by bond breaking between the β-carbon and oxygen atom (alkyl cleavage), which could lead to either inversion of configuration, path b, or racemization.[12]

Scheme I



The optical rotations of bacterial P[(*R*)-HB] and those of the polymers synthesized by the ring-opening polymerization reactions of (*S*)-BL were used to determine the predominant stereochemical configurations of the asymmetric centers in the polymer repeating units. In addition, the isomeric purities of the repeating units were also determined by controlled, acid-catalyzed methanolysis to obtain the constituent methyl β-hydroxybutyrate stereoisomer in the polymers.[13] The isotactic (i) and syn-

† Present address: Department of Chemistry, University of Lowell, Lowell, MA 01854.

diotactic (s) diad sequences were also obtained from ^{13}C NMR spectra of PHB to determine the stereochemical sequence distributions of the repeating units.[14,15]

Three catalysts, which were investigated in this work, were prepared by the reaction of either triethylaluminum ($AlEt_3$) or diethylzinc ($ZnEt_2$) with water.[15-18] Two different aluminum-based catalysts were evaluated as follows: (1) a catalyst prepared in situ by the low-temperature reaction of $AlEt_3$ and water in toluene[15] and (2) a prepurified catalyst, which contained an oligomeric ethylaluminoxane structure, EAO.[15,18] The zinc-based catalyst was prepared using a $ZnEt_2/H_2O$ ratio of 1/0.6.[18]

Experimental Section

^1H NMR Spectroscopy. Proton (^1H) nuclear magnetic resonance (NMR) spectra were taken on a Varian XL-200 spectrometer, and infrared spectra (IR) were recorded on a Perkin-Elmer Model 283 spectrometer. ^1H NMR chemical shifts in parts per million (ppm) are reported downfield from the internal standard tetramethylsilane at 0.00 ppm. The spectral position of sample IR absorbances are given in units of reciprocal centimeters. Peak areas for ^1H NMR spectra were determined by spectrometer integration and are reported as relative intensities representing a given number of hydrogens. The following abbreviations are used to present the ^1H NMR spectral results: s = strong, vs = very strong, m = medium, br = broad, sh = shoulder, sha = sharp, db = doublet.

^{13}C NMR Spectroscopy. Solution ^{13}C NMR measurements of the polymer samples were recorded at 75.4 MHz on a Varian XL-300 NMR spectrometer. The ^{13}C NMR spectra were recorded at 20 °C using chloroform-*d* as both the solvent (40 mg of polymer/mL) and the internal chemical shift reference. The delay time between sampling pulses was 3.0 s. The spectra taken were proton-decoupled with a 16 500-Hz spectral width, 30K data points, and a 56° pulse (10 μs), and typically 5000–10 000 transients were accumulated.

Molecular Weight Measurements. All molecular weights reported were determined by GPC using a Waters Model 6000A solvent delivery system, Model 401 refractive index detector, and Model 730 data module with 2 Ultrastyragel linear columns in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 0.3% w/v and injection volumes of 300 μL were used. Polystyrene standards with a low polydispersity were purchased from Polysciences and used to generate a calibration curve.

Elemental Analyses. Analyses were carried out by the Microanalysis Laboratory at the University of Massachusetts, Amherst, MA. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter in a temperature-equilibrated cell.

Optical Rotation. Optical rotation data are reported as follows: $[\alpha]^{\circ}C_{\lambda_{nm}}$ = specific rotation (concentration in g/100 mL of solvent).

All of the synthetic procedures described below, with the exception of the lactonization to prepare (*S*)-BL, were carried out under argon with the transfer of all liquids carried out under inert atmosphere with syringes through septum caps.

(*R*)-Methyl β-Hydroxybutyrate (1). The procedure followed, which involved the acidic methanolysis of P[(*R*)-HB], was the same as previously described in the literature.[13,19] This compound was a colorless liquid: $[\alpha]^{25}_{589} = -49.6°$ (1.3, $CHCl_3$) [lit.[19] $[\alpha]^{22}_{589} = -48.6°$ (1.3, $CHCl_3$)].

(*R*)-Benzyl β-Hydroxybutyrate (2).[20] Into a single-neck flask was transferred 52 mL (0.5 mol) of benzyl alcohol (vacuum distilled from CaH_2) and 11.7 g (0.10 mol) of 1. *p*-Toluenesulfonic acid (Aldrich Chemical Co.; monohydrate, 99% purity; 95 mg 5×10^{-4} mol) was added to the flask by removing the septum cap and adding the solid while flushing with argon. The flask was fitted with a distillation apparatus in which the receiving flask was cooled to 0 °C. The reaction flask was heated to 80 °C with stirring according to the following sequence: (1) 4.5 h under an argon atmosphere, (2) 2 h at 160 mm, (3) 2 h at 140 mm, and (4) 5 h at 100 mm. The reaction contents were combined with diethyl ether (300 mL) and extracted sequentially with a saturated aqueous $NaHCO_3$ solution (30 mL), a 5% aqueous $NaHCO_3$ solution (2 × 40 mL), and brine (3 × 40 mL). The solution was dried with $MgSO_4$, and ether and benzyl alcohol were removed on a rotary evaporator [30 °C (150 mmHg)] and by use of vacuum distillation [50 °C (0.2 mmHg)], respectively. The remaining crude oily product was purified by vacuum distillation (90 °C, 10^{-3} mm) to obtain 18.1 g (94% yield) of a colorless clear liquid: $[\alpha]^{25}_{589} = -31.08°$ (5.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) 1.21 (d, 3 H), 2.50 (m, 2 H), 4.25 (m, 1 H), 5.15 (s, 2 H), 7.35 (s, 5 H); IR (neat liquid, NaCl plates) 3400 (s, br), 3050 (m, db), 2900 (s, sh), 1710 (vs, sha), 1160 (s, sha), 720 (s, db). Elem anal. Obsd: C, 67.79; H, 7.35. Calcd: C, 68.02; H, 7.27.

(*R*)-Benzyl β-*O*-Mesylbutyrate (3). Dry pyridine (15.8 mL, 0.19 mol), which was distilled from CaH_2, and mesyl chloride (9.7 mL, 0.13 mol), which was obtained from Aldrich Chemical Co. and used as received, were added to a single-neck flask and kept at 25 °C for 15 min, followed by 30 min at −15 °C, after which time it had developed a deep red-brown color. To the contents of the flask was added a CH_2Cl_2 solution (100 mL, distilled from P_2O_5) containing 17.9 g (0.09 mol) of **2** cooled to −15 °C. The reaction mixture was maintained at approximately 5 °C for 40 h and then at 25 °C for 5 h under an argon atmosphere. After the reaction period, the solution was brown in color, and the flask contained a crystal precipitate. The solution was gravity filtered into a separatory funnel, diluted with 450 mL of diethyl ether, and extracted sequentially with 1 N HCl (2 × 60 mL), 0.1 N HCl (4 × 90 mL), 5% aqueous $NaHCO_3$ (3 × 90 mL), and H_2O (2 × 90 mL). After the solution was dried with $MgSO_4$, the solvent was removed to give 24.6 g (98% yield) of a clear, yellow oil: $[\alpha]^{25}_{589} = -19.25°$ (5.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) 1.48 (d, 3 H), 2.70 (m, 2 H), 2.90 (s, 3 H), 5.13 (s, 2 H; m, 1 H), 7.35 (s, 5 H); IR (neat liquid, NaCl plates) 3050 (m, sha), 2900 (m, db), 1730 (vs, sha), 1350 (vs, br), 1170 (s, br), 910 (s, db), 720 (s, db). Elem anal. Obsd: C, 53.29; H, 5.99. Calcd: C, 52.93; H, 5.92.

(*R*)-β-*O*-Mesylbutyric Acid (4). Into a single-neck flask were transferred 23.7 g (0.087 mol) of **3**, 38 mL of a 30–32 wt % HBr in glacial acetic acid solution (Aldrich Chemical Co.), and 8 mL of methylene chloride (spectroscopic grade, Aldrich Chemical Co.). The reaction was carried out with stirring at 25 °C for 6 h, after which the reaction contents were maintained at −15 °C for 16 h. The solvent was removed by heating to 45 °C under reduced pressure, by first using a water aspirator (approximately 100 mmHg) and then a vacuum pump (20 mmHg) to leave a crude brown oil. Continued heating of this oil at 45 °C resulted in degradation of the product. Flash chromatography on 100 g of 230–400-mesh silica gel using chloroform (300 mL) followed by chloroform/methanol (8/2) eluent gave an elution volume of 385–865 mL, which, after solvent evaporation, gave 11.3 g (71% yield) of a light brown liquid: $[\alpha]^{25}_{589} = -38.45°$ (5.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) 1.48 (d, 3 H), 2.75 (m, 2 H), 3.00 (s, 3 H), 5.10 (m, 1 H); IR (neat liquid, NaCl plates) 3300 (s, br), 2990 (s, br), 2600 (m, br), 1710 (vs, sha), 1330 (vs, sh), 1160 (s, sha), 900 (s, sha). Elem anal. Obsd: C, 33.24; H, 5.56. Calcd: C, 32.97; H, 5.53.

(*S*)-β-Butyrolactone [(*S*)-BL].[13,21-23] To a beaker were added 20.4 g (0.11 mol) of **4** and 260 mL of H_2O. The mixture was cooled to 5 °C, and $NaHCO_3$ (11.0 g, 0.13 mol) was added slowly with stirring so that the pH obtained was approximately 7.5. This solution was extracted with diethyl ether (50 mL) in a separatory funnel, and the aqueous layer and methylene chloride (260 mL) were transferred into a three-neck flask fitted with an overhead stirrer and a condenser. The two-phase mixture was maintained at 35 °C for 2 h with rapid stirring. The two layers were then separated, the aqueous layer was returned to the three-neck flask, a second portion of methylene chloride (260 mL) was added, and the reaction was continued for an additional 2 h, at 35 °C, with rapid stirring. The organic phase from the first 2-h reaction period was extracted with 5% aqueous $NaHCO_3$ (2 × 120 mL) and H_2O (3 × 120 mL). The above extraction procedure was repeated for the methylene chloride phase from the second 2-h reaction period. The two organic solutions from the extractions were combined and dried over $MgSO_4$, and the solvent was removed leaving a clear, yellow liquid, which was distilled in a short path distillation apparatus [bp 70 °C (30 mmHg)] to obtain 4.24 g (45% yield) of a

colorless liquid: $[\alpha]^{25}_{589} = -26.1°$ (5.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) 0.85 (d, 3 H), 2.28 (m, 2 H), 3.82 (m, 1 H); IR (neat liquid, NaCl plates) 2980 (m, db), 1820 (vs, sha), 1110 (s, sha), 1005 (m, sha), 800 (m, sha). Elem anal. Obsd: C, 55.20; H, 7.05. Calcd: C, 55.81; H, 7.03. Immediately before polymerization (*S*)-BL was dried over CaH_2 with stirring at room temperature for 16 h followed by an additional short path distillation performed as described above.

Catalyst Preparations. The $ZnEt_2/H_2O$ (1/0.6) catalyst preparation is similar to that previously described in the literature.[18] All glassware used was flame dried under vacuum, and transfers were carried out under an argon atmosphere either by use of a syringe or in a drybox. Into a 50-mL round-bottom flask were added 15 mL of dry 1,4-dioxane (distilled from sodium metal) and 3.5 mL (3.42×10^{-2} mol) of $ZnEt_2$ (purchased from Aldrich Chemical Co.). This solution was cooled to 0 °C, and 0.37 mL (2.05×10^{-2} mol) of deoxygenated, distilled H_2O was added dropwise with stirring under argon over a 15-min period. After an additional 1-h reaction period with stirring at 25 °C, the volatiles were removed [25 °C (10^{-1} mmHg)], leaving 1.5 g of a yellow powder. To 0.5 g of this yellow powder was added 16 mL of dry toluene (distilled from sodium metal), giving a clear yellow catalyst solution above an insoluble yellow powder.

The in situ $AlEt_3/H_2O$ (1/1) catalyst and the EAO catalyst were prepared as previously described.[15] A determination of the $\bar{M}_n$ value for the EAO catalyst was carried out using a Knauer Model 11 vapor pressure osmometer (VPO) in a drybox, and all transfers and manipulations were made in the drybox. The instrument was calibrated with benzil, and the solvent used was dry benzene. The value of $\bar{M}_n$ of the EAO catalyst was 16.

Polymer Preparations. The polymerization of (*S*)-BL with the in situ $AlEt_3/H_2O$ (1/1) and EAO catalysts was performed as previously described for the polymerization of (*R,S*)-BL.[15]

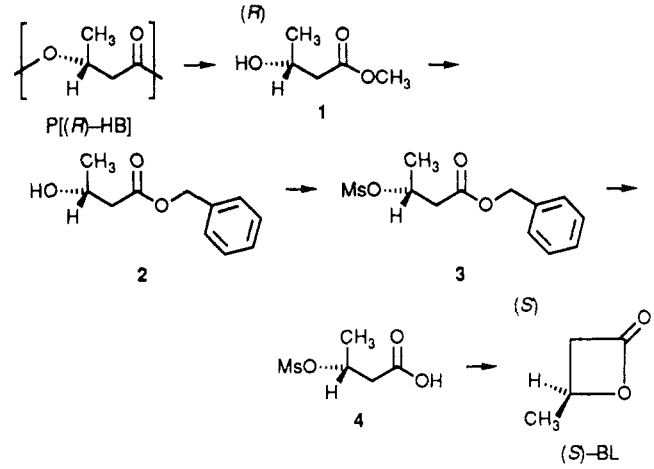
The polymerization reactions of (*S*)-BL and (*R,S*)-BL with the $ZnEt_2/H_2O$ catalyst were carried out in flame dried (in vacuo) glassware by transferring all reactant through septum caps with a syringe under an argon atmosphere. The clear yellow initiator solution (1.0 mL) was added to the polymerization ampule, which was cooled in a liquid nitrogen bath. Both (*S*)-BL and (*R,S*)-BL (1.0 g, 1.2×10^{-2} mol), which were purified by distillation from CaH_2, were further degassed by three freeze–thaw cycles, and the polymerization ampule was sealed under vacuum at room temperature. The polymerization reactions were carried out at 60 °C for 5 days.

The purification procedure for all polymers prepared was identical with that previously described for the polymerization of racemic BL with aluminum-based catalysts.[15] Both residual aluminum and zinc were removed effectively from the polymer samples by using acetylacetone (AcAc) as previously described,[15] so that the amount of aluminum or zinc remaining in the final polymer products was less than 0.1% as determined by microanalysis.

Polymer Fractionation. The polymer obtained from (*S*)-BL using the EAO catalyst was fractionated in the following manner. To the polymer sample dissolved in $CHCl_3$ (0.25 wt %) was slowly added methanol, at room temperature, with vigorous stirring, until the cloud point was reached. The resulting polymer precipitate was collected by gravity filtration to give the high molecular weight fraction (30 wt %). Additional methanol was then added to the filtrate to provide a second polymer precipitate, which is designated as the middle molecular weight fraction (50 wt %). The solvent from the final filtrate was then removed by rotoevaporation, and the remaining solid was dissolved in chloroform and precipitated in methanol to give the low molecular weight fraction (20 wt %).

Polymer Methanolysis. In a Pyrex culture tube with a Teflon-lined screw cap surrounded by stainless steel wire mesh was dissolved a polymer sample (0.1 g) in dry $CHCl_3$ (15 mL). To this solution was added under an argon atmosphere 15 mL of a 3% by volume H_2SO_4 in methanol solution. This mixture was heated in an external oil bath at 100 °C for 4 h. Subsequently, half-saturated aqueous NaCl solution (3 mL) and diethyl ether (15 mL) were added, and this mixture was transferred to a separatory funnel. The two layers were separated, and the aqueous layer was extracted with ether. The organic phase and the ether extract were combined and washed with a saturated aqueous $NaHCO_3$ solution and a saturated aqueous NaCl solution sequentially and dried over $MgSO_4$, and the solvent was removed under reduced pressure.

Scheme II



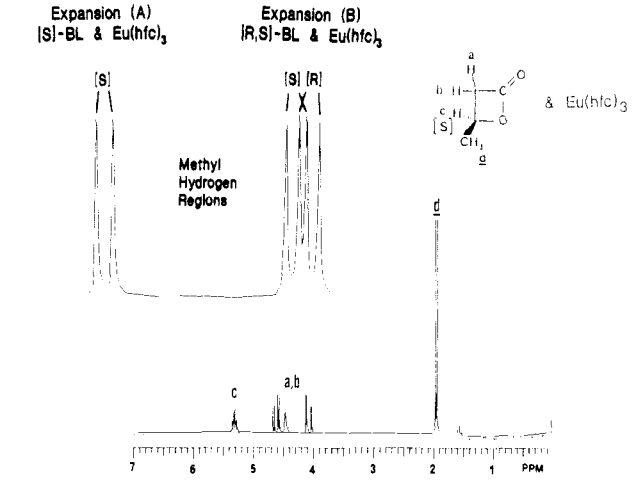


Figure 1. 200-MHz ^1H NMR spectrum, recorded at 25 °C in CCl_4/benzene-d_6 (90/10), of (*S*)-BL complexed with 30 mol % $Eu(hfc)_3$; insets are expansions of the methyl regions for both this monomer, A, and (*R,S*)-BL, B.

Results

Monomer Synthesis. The preparation of (*S*)-BL was carried out in five steps as shown in Scheme II from a readily available chiral precursor methyl (*R*)-β-hydroxybutyrate, which was obtained from poly[(*R*)-HB] produced by bacteria,[11] in an overall yield of 20%.

The optical purity of (*S*)-BL so obtained was determined by comparison of its ^1H NMR spectra with that of (*R,S*)-BL with both complexed to the chiral shift reagent tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium (Aldrich Chemical Co.). In Figure 1, the ^1H NMR spectrum of (*S*)-BL in the presence of 30 mol % of the shift reagent is shown along with expansions of the methyl hydrogens (d) from the spectra of both (*S*)-BL and (*R,S*)-BL so complexed.

Expansion B in Figure 1 contains two doublets. The higher field doublet was assigned by analyzing a mixture of BL stereoisomer to the d hydrogens of the (*R*) enantiomer as indicated in Figure 1. Expansion A appears to be a single doublet. From this analysis, it was determined that the sample of (*S*)-BL so prepared had an optical purity in excess of 97%.

The yield and molecular weights of the polymers obtained from the polymerization of (*S*)-BL with the zinc-

Table I
Ring-Opening Polymerization of (S)-BL

catalyst	yield, %	$\bar{M}_w$[a]	$\bar{M}_w/\bar{M}_n$[a]
$ZnEt_2/H_2O$ (1/0.6)	72	20 000	1.5
in situ $AlEt_3/H_2O$ (1/1)	30	240 000	8.0
EAO[b] $[-AlEtO-]_n$	50	190 000	7.8

[a] Determined by GPC analysis of chloroform solutions at 25 °C. [b] EAO is ethylaluminoxane.

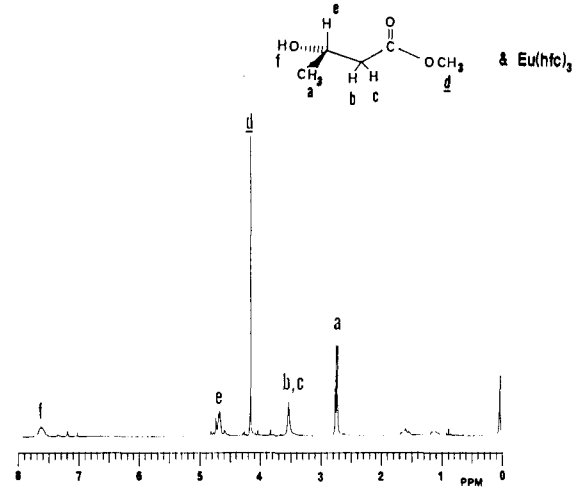


Figure 2. 200-MHz ^1H NMR spectrum, recorded at 25 °C in CCl_4/benzene-d_6 (90/10), of (S)-methyl β-hydroxybutyrate, which was obtained by the methanolysis of the polymer sample obtained with $ZnEt_2/H_2O$ catalyst from (S)-BL, complexed with 20 mol % $Eu(hfc)_3$.

and aluminum-based catalyst systems are given in Table I.

The stereoisomeric purities of the repeating units of each of the polymers were determined by an acid-catalyzed methanolysis of the polymers to form the constituent methyl β-hydroxybutyrate stereoisomers. The ^1H NMR spectrum obtained in the presence of 20 mol % of the shift reagent of the methanolysis products obtained from the polymer obtained with the $ZnEt_2/H_2O$ (1/1) catalyst is shown in Figure 2. Expansions of the peaks for the hydrogen atoms in the methoxy group, which are designated as d, in order to determine stereoisomeric purity of the methyl esters, are displayed in Figure 3.

In Figure 3a an expansion is shown of the d hydrogen signals from the spectrum of methyl (R,S)-β-hydroxybutyrate, which was obtained from the methanolysis of an (R,S)-P(HB) sample and complexed with the shift reagent. The upfield signal was assigned to the (R) stereoisomer from an isomeric mixing experiment. From curve fitting approximations for Figure 3c and from spectrometer integration of Figure 3d, the isomeric purities of the repeating units were determined, as shown in Table II.

The specific rotations ($[\alpha]^{30}_{365}$) for all polymers, along with that of bacterial P[(R)-HB], are given in Table II. From the above stereochemical analysis of the PHB products, the apparent mode of (S)-BL ring opening for each of the catalyst studied was determined, and the results are given in Table II.

Because of the relatively lower specific rotation of the polymer obtained with the EAO catalyst (see Table II), a further study of this sample was undertaken. The stereochemical diad sequence distribution was determined by ^{13}C NMR spectroscopy for all four polymers of Table II. A typical spectrum, that of the polymer obtained from (S)-BL using the $ZnEt_2/H_2O$ (1/0.6) catalyst, is shown in Figure 4. Expansion of the carbonyl region of the spectrum in Figure 4 is shown in Figure 5a, and similar expansions of the ^{13}C NMR spectra taken under the identical conditions of an essentially random stereocopolymer from racemic BL, which was polymerized with the $ZnEt_2/H_2O$ (1/0.6) catalyst, are shown in Figure 5b, while that of the polymer obtained from (S)-BL using the EAO catalyst is shown in Figure 5c. In Figure 5b, the isotactic (i) and syndiotactic (s) diads were assigned, as was previously described.[15] Only one peak is observed in each of the expansions shown in parts a and c of Figure 5, and the diad assignments for both were previously determined.[15]

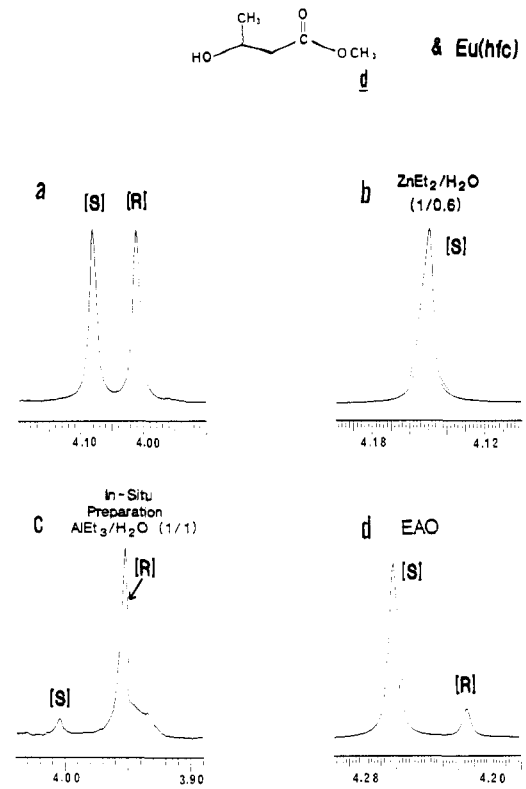


Figure 3. Expansions of the methyl ester hydrogen region from the ^1H NMR spectra, recorded in the presence of $Eu(hfc)_3$ as described in Figure 2, of methyl β-hydroxybutyrate stereoisomers obtained from the methanolysis of (a) the polymer obtained from racemic BL using the $ZnEt_2/H_2O$ catalyst, (b) the polymer obtained from (S)-BL using the $ZnEt_2/H_2O$ catalyst, (c) the polymer obtained from (S)-BL using the in situ $AlEt_3/H_2O$ (1/1) catalyst preparation, and (d) the unfractionated polymer obtained from (S)-BL using the EAO catalyst preparation.

The polymer obtained from (S)-BL using the EAO catalyst was fractionated by solvent extraction, and the molecular weights and specific rotations of the polymer fractions obtained are presented in Table III and in the GPC chromatograms in Figure 6.

Discussion

Monomer Synthesis. (R)- and (S)-BL were previously prepared through the diastereomeric salts formed from (R,S)-β-bromobutyric acid and α-(1-naphthyl)ethylamine to form either (R)- or (S)-β-bromobutyric acid. The β-bromobutyric acids were then converted to their respective lactones to obtain samples of the desired stereochemistry.[21,22] Optically active BL, synthesized in this manner, had optical purities ranging from 73%[22] to greater than 97%. Seebach and co-workers recently reported a synthetic route to (S)-BL with an optical purity of greater than 98% using the chiral precursor poly[(R)-HB].[13] This synthetic route involves the pyrolysis of a cyclic orthocarbonate, which degrades thermally forming the corresponding β-lactone.[13]

Table II
Stereochemical Analysis of Synthesized and Natural Origin PHB Samples

catalyst	confign of BL	repeating unit stereochemistry % (*R*)/% (*S*)[a]	$[\alpha]^{30}_{365}$ (3.1, $CHCl_3$) (±0.4°)	apparent mode of ring opening
Alcaligenes[b] *eutrophus*		100/0	+7.4°	
$ZnEt_2/H_2O$ (1/0.6)	(*S*)	0/100	−7.0°	path a with retentn of confign
in situ $AlEt_3/H_2O$ (1/1)	(*S*)	93/7[c]	+6.9°	path b with inversn of confign[d]
EAO[e]	(*S*)	15/85	−5.8°	both path a and b operative

[a] Determined by methanolysis of the polymer samples to their corresponding methyl β-hydroxyalkanoate (see the Experimental Section) followed by NMR analysis in the presence of $Eu(hfc)_3$ (see the Results). [b] Sample obtained from Aldrich Chemical Co.; GC analysis of the methyl ester degradation products showed exclusively HB repeating units. [c] The value of 7% for the mol % of (*S*) repeating units is a maximum value based on the assumption that the lower field peak in Figure 5c is not due to an impurity (see ref 28). [d] From the present study, the possibility of a small degree, not exceeding 7%, of ring opening occurring by path a cannot be excluded. [e] EAO is ethylaluminoxane.

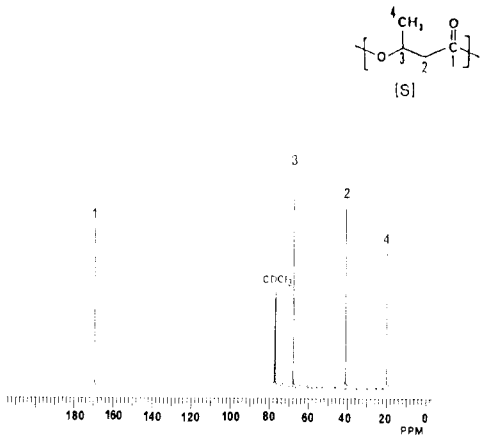


Figure 4. 75.4-MHz ^{13}C NMR spectrum, recorded at 25 °C in $CDCl_3$, of P[(*S*)-HB] obtained from (*S*)-BL using the $ZnEt_2/H_2O$ catalyst.

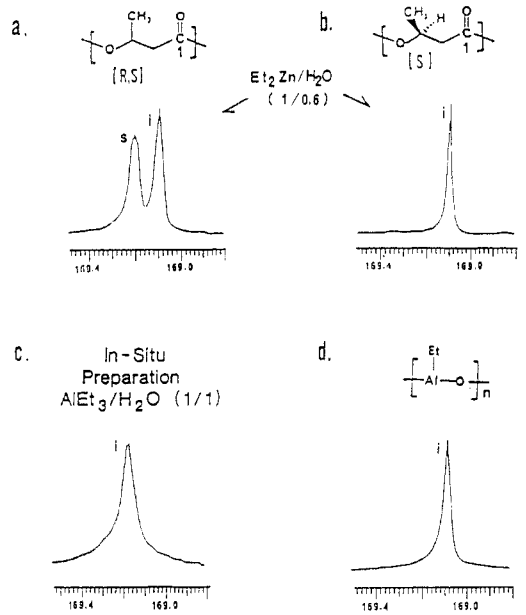


Figure 5. Expansions of the carbonyl carbon region from the ^{13}C NMR spectra, recorded at 25 °C in $CDCl_3$, of (a) the polymer obtained from racemic BL using the $ZnEt_2/H_2O$ catalyst, (b) the polymer obtained from (*S*)-BL using the $ZnEt_2/H_2O$ catalyst, (c) the polymer obtained from (*S*)-BL using the in situ catalyst, and (d) the polymer obtained from (*S*)-BL using the EAO catalyst preparation.

In this report, the synthetic scheme in Scheme II was used to obtain (*S*)-BL in gram quantities with a high optical purity (more than 97%). The high optical purity of (*S*)-BL was essential for the unambiguous interpretation of the stereochemical studies carried out in this study.

Polymerization with the $ZnEt_2/H_2O$ (1/0.6) Catalyst. The polymerization of (*S*)-BL when carried out as described in the Experimental Section by using a $ZnEt_2/H_2O$ (1/0.6) catalyst system proceeded by path a with retention of configuration and little or no racemization. Using this method, we have prepared the enantiomer of natural origin PHB, P[(*S*)-HB].

The results are consistent with the work of Le Borgne and co-workers, who studied the stereoselective polymerization of racemic BL using a related Zn-based catalyst system, $ZnEt_2$/(*R*)-(−)-3,3-dimethyl-1,2-butanediol ((*R*)-(−)-DMBD). These workers found the unreacted monomer was enriched in the (*S*) enantiomer and that the polymer obtained had repeating units that were predominantly of the (*R*) chirality. Therefore, it was concluded by Le Borgne and co-workers that ring opening using the Zn/(*R*)-(−)-DMBD catalyst proceeded primarily with retention of configuration.

Table III
Characterization of the Various Molecular Weight Fractions[a] from the PHB Sample Synthesized by the EAO-Catalyzed Polymerization of (*S*)-BL

fraction	$\bar{M}_w$[b]	$\bar{M}_w/\bar{M}_n$[b]	wt % of the fraction[c]	$[\alpha]^{30}_{365}$ (3.1, $CHCl_3$) (±0.4°)
unfractionated sample	240 000	17	100	−5.8°
high MW	430 000	14	30	−3.6°
middle MW	20 000	1.5	50	−7.2°
low MW	8 000	1.3	20	−7.1°

[a] Fractionation was carried out by solubility differences (see the Experimental Section). [b] Determined by GPC analysis of chloroform solutions at 25 °C. [c] Relative to the weight of the unfractionated sample.

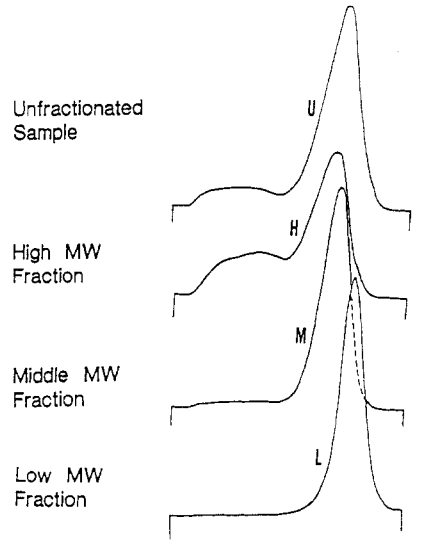


Figure 6. GPC chromatograms, recorded at 25 °C in $CHCl_3$, of PHB using the EAO catalyst and measured before and after fractionation.

Polymerization with the $AlEt_3/H_2O$ In Situ Catalyst. The polymerization of (S)-BL when carried out as previously described,[15] using the $AlEt_3/H_2O$ (1/1) in situ catalyst system, proceeded primarily by path b, with inversion of configuration and little to no racemization (see Table II), producing a polymer that contained greater than 93% (R)-HB repeating units. Therefore, by proper choice of the appropriate catalyst system, PHB could be synthesized from (S)-BL in either desired stereochemistry, (R) or (S).

A mechanistic study of the ring-opening polymerization of (R)-BL with an $AlEt_3/H_2O$ catalyst was initially carried out by Shelton and co-workers.[20,21] The monomer used in their work had an enantiomeric excess equal to 73% of the (R) configuration. The catalyst used was an in situ $AlEt_3/H_2O$ (1/1) preparation. From a comparison of the chiroptical properties for the polymer synthesized to that of natural origin P[(R)-HB], they concluded that the mode of ring opening proceeded by path a with retention of configuration. Their results, therefore, would seem to be in contradiction with the findings obtained in the present study with an $AlEt_3/H_2O$ (1/1) in situ catalyst system.

The different mechanisms of ring opening observed in the present study and from the work of Shelton and co-workers[20,21] for the in situ $AlEt_3/H_2O$ (1/1) preparation need further consideration. The ring-opening polymerizations of β-monosubstituted β-propiolactones using $AlEt_3/H_2O$ catalysts are apparently extremely sensitive to the precise experimental conditions used for the catalyst preparation. In a previous study with a different β-lactone monomer we prepared the catalyst by adding $AlEt_3$ in toluene (1.9 M) to the monomer at −78 °C followed by the addition of H_2O ($AlEt_3/H_2O$, 1/1), while maintaining the temperature at −78 °C, and then slowly warming. From this procedure, a noncrystalline polymer was obtained in 60% yield with an $\bar{M}_w$ of approximately 20 000, and an $\bar{M}_w/\bar{M}_n$ of less than 2.0 was obtained. When the polymerization of that lactone was carried out in the same manner as in the present study in which the catalyst was prepared first in the polymerization ampule before the addition of monomer,[15] the polymer product obtained was dramatically different. In that case, the polymer produced was crystalline but was obtained in only a 12% yield and had an $\bar{M}_w$ of approximately 400 000 and an $\bar{M}_w/\bar{M}_n$ of approximately 8.0.[15] These results clearly show how sensitive the polymerization reaction is to the procedure used to prepare the $AlEt_3/H_2O$ catalyst.

Indeed, the polymerization of enantiomerically enriched BL by Shelton and co-workers was carried out by preparation of the catalyst in the presence of monomer,[20] while in the present study the in situ preparation of $AlEt_3/H_2O$ was carried out first before the addition of monomer.[15] The differences in the methods above undoubtedly account for the different modes of ring opening observed. It is likely, therefore, that by careful alteration of the method used to prepare an $AlEt_3/H_2O$ catalyst the mode of the ring-opening reaction can be changed completely to achieve any desired stereochemistry in the polymer. This point is again illustrated from the results discussed below, which describe the polymer stereochemistry obtained when the EAO catalyst preparation was used to polymerize (S)-BL.

Polymerization with the EAO Catalyst. The polymerization of (S)-BL with the EAO catalyst[15] proceeded primarily by path a with retention of configuration, although the magnitude for the specific rotation of the product was less than that of natural origin PHB (see Table II). The PHB produced contained 85% (S) repeating units (see Table II). The lower isotopic purity of the repeating units relative to the starting monomer was not due to racemization of the monomer before its polymerization as shown by removal of the nonpolymerized monomer from the reaction mixture and characterization by ^1H NMR in the presence of $Eu(hfc)_3$. The unreacted lactone had an optical purity in excess of 97%.

Fractionation of the PHB obtained gave middle and low molecular weight fractions having specific rotations that were equal in magnitude within experimental error but opposite in sign to the value for natural origin PHB (see Tables II and III). This results suggests that for the EAO catalyst there exist active sites that produce relatively lower molecular weight chains by the ring-opening polymerization of BL by path a with little or no racemization.

The high molecular weight fraction was still bimodal and contained a relatively much higher molecular weight polymer, which corresponded to the high molecular weight GPC peak of the unfractionated polymer (see Figure 6). This fraction had a specific rotation with a much smaller value but of the same sign as the middle and low molecular weight fractions (see Table III).

The expanded carbonyl region of the ^{13}C NMR spectrum (75.4 MHz) of the unfractionated PHB sample produced using the EAO catalyst is shown in Figure 5c. From the single peak in Figure 5c it is clear that the polymer produced from (S)-BL using the EAO catalyst did not contain measurable (from ^{13}C NMR) quantities of syndiotactic diads. The single peak in Figure 5c in combination with the specific rotations of the unfractionated polymer sample and the high molecular weight fraction suggests that either very long blocks or (less likely) whole chains contained only (R) units in the high molecular weight fraction along with a larger amount of polymer that contained exclusively (S) repeating units.

It is interesting to note that the polymerization of racemic BL with the EAO catalyst produced both a relatively high molecular weight, highly crystalline fraction and a lower molecular weight fraction that showed little crystallinity,[15,18] which suggests that the EAO catalyst has at least two, very different types of active sites.[15] It would not be surprising, therefore, if these different active sites operated by different ring-opening mechanisms, although the major mode of ring opening apparently proceeds by path a with retention of configuration. Similarly, Kricheldorf and co-workers found that aluminum triisopropoxide is also capable of polymerizing BL by path a.[26]

Acknowledgment. We are grateful for the financial support received from the Center for the University of Massachusetts Industry Research on Polymers (CUMIRP) and the Office of Naval Research, Molecular Biology Program (Grant N00014-86-K-0369).

References and Notes

(1) Dawes, E. A. *Microbial Energetics*; Chapman and Hall: New York, 1986; Chapter II. Dawes, E. A.; Senior, P. J. *Adv. Microb. Physiol.* **1973**, *10*, 135–266.
(2) Findlay, R. H.; White, D. C. *Appl. Environ. Microbiol.* **1983**, *45*, 71.
(3) Capon, R. J.; Dunlop, R. W.; Ghisalberti, E. L.; Jefferies, P. R. *Phytochemistry* **1983**, *22*, 1181.
(4) Wallen, L. L.; Rohwedder, W. K. *Environ. Sci. Technol.* **1974**, *8*, 576.
(5) Bloembergen, S.; Holden, D. A.; Bluhm, T. L.; Hamer, G. K.; Marchessault, R. H. *Macromolecules* **1987**, *20*, 3086. Bloem-

bergen, S.; Holden, D. A.; Marchessault, R. H. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, *29* (1), 594.
(6) Gross, R. A.; DeMello, C.; Lenz, R. W.; Brandl, H.; Fuller, R. C. *Macromolecules* **1989**, *22*, 1106.
(7) Brandl, H.; Gross, R. A.; Lenz, R. W.; Fuller, R. C. *Appl. Environ. Microbiol.* **1988**, *54*, 1977.
(8) Gross, R. A.; Brandl, H.; Ulmer, H. W.; Posada, M. A.; Fuller, R. C.; Lenz, R. W. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1989**, *30* (1), 492.
(9) Brandl, H.; Gross, R. A.; Knee, E. J.; Lenz, R. W.; Fuller, R. C. *Int. J. Appl. Biol.* **1989**, *11*, 49.
(10) Doi, Y.; Kunioka, M.; Tamaki, A. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, *29* (1), 588.
(11) Holmes, P. A. *Phys. Technol.* **1985**, *16*, 32.
(12) Lavallee, C.; Grenier, D.; Prud'homme, R. E.; Le Borgne, A.; Spassky, N. In *Advances in Polymer Synthesis*; Culbertson, B. M., McGrath, J. E., Eds.; Plenum: New York, 1985; p 450.
(13) Seebach, D.; Roggo, S.; Zimmermann, J. In *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH: Weinheim, West Germany, 1987; Vol. 17, pp 7-126.
(14) Iida, M.; Hayase, S.; Araki, T. *Macromolecules* **1978**, *11*, 490.
(15) Gross, R. A.; Zhang, Y.; Konrad, G.; Lenz, R. W. *Macromolecules* **1988**, *21*, 2657.
(16) Agostini, D. E.; Lando, J. B.; Shelton, J. R. *J. Polym. Sci., Part A-1* **1971**, *9*, 2775.
(17) Teranishi, K.; Iida, M.; Araki, T.; Yamashita, S.; Tani, H. *Macromolecules* **1974**, *7*, 421 and references therein.
(18) Iida, M.; Araki, T.; Teranishi, K.; Tani, H. *Macromolecules* **1977**, *10*, 275.
(19) Le Borgne, A.; Spassky, N. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1988**, *29* (1), 598.
(20) Shelton, J. R.; Lando, J. B.; Agostini, D. E. *J. Polym. Sci., Part B* **1971**, *9*, 173.
(21) Shelton, J. R.; Agostini, D. E.; Lando, J. B. *J. Polym. Sci., Part A-1* **1971**, *9*, 2789.
(22) Kricheldorf, H. R.; Berl, M.; Scharnagl, N. *Macromolecules* **1988**, *21*, 286.

Morphological Characterization of Bioerodible Polymers. 1. Crystallinity of Polyanhydride Copolymers

Edith Mathiowitz, Eyal Ron, George Mathiowitz,† Carmela Amato, and R. Langer*

Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received October 6, 1989; Revised Manuscript Received December 20, 1989

ABSTRACT: The composition of polyanhydrides has been a critical determinant in enabling different erosion rates of these polymers. Since crystallinity is an important factor in controlling polymer erosion rates, an in-depth analysis of the effect of polymer composition on crystallinity was undertaken using a variety of polyanhydrides. Polyanhydride homopolymers and copolymers made of sebacic acid (SA), (carboxyphenoxy)propane (CPP), (carboxyphenoxy)hexane (CPH), and fumaric acid (FA) were studied. Crystallinity was analyzed by the following: (1) X-ray diffraction, (2) a combination of X-ray diffraction and differential scanning calorimetry (DSC), and (3) data generated from ^{1}H NMR spectroscopy and Flory's equilibrium theory. Homopolymers such as poly(sebacic anhydride), poly(fumaric anhydride), and poly(1,3-bis(*p*-carboxyphenoxy)propane anhydride) were crystalline, and each displayed a typical powder diffraction. In copolymers, diffraction patterns were determined, in most cases, by the monomer of highest concentration. Copolymers with high ratios of SA or CPP had a high crystallinity while copolymers with almost equal ratios of SA and CPP or SA and CPH were amorphous. The poly(SA-FA) series displayed high crystallinity regardless of monomer concentration.

Introduction

Bioerodible polymers have been used in a number of applications including biomaterials and drug carriers. The degradation process in these polymers is generally classified as either bulk or surface erosion. Surface erosion is achieved when the degradation occurs at the surface of the device and approaches the center in a predictable way. In contrast, bulk erosion is characterized by degradation that occurs simultaneously throughout the device. The type of erosion obtained is influenced by the intrinsic chemical properties of the polymer such as hydrophobicity and bond stability. Morphological characteristics of the polymer are also important factors in controlling erosion rates. Crystalline regions erode more slowly than amorphous regions,[1,2] and the type of crystals that form the crystalline region may affect the erosion rate. In polyanhydrides, the model polymer for this study, chemical properties such as bond stability, hydrophobicity, and degradation rate have been previously examined only to a limited extent.[3,4] A quantitative correlation between polyanhydride copolymer composition and device morphology has yet to be established.

In this paper we report the effect of copolymer composition on certain aspects of polyanhydride morphology. Polymers were characterized by differential scanning calorimetry (DSC) to yield thermal parameters such as glass transition temperature (T_g) and the melting point (T_m). Crystallinity was characterized with three different methods: (1) X-ray diffraction, (2) a combination of

* All correspondence should be addressed to Dr. R. Langer at MIT.
† Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115.

0024-9297/90/2223-3212$02.50/0