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Crystal Structure of Syndiotactic Poly(β-hydroxybutyrate) from X-ray Fiber and Powder Diffraction Analyses and Molecular Modeling

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ABSTRACT: A preliminary crystal structure of syndiotactic poly(β-hydroxybutyrate), syn PHB, was derived by using X-ray fiber and powder diffraction data in conjunction with computational modeling. The X-ray fiber diagram of highly stretched 69% syn PHB gave 23 reflections and a fiber repeat of 7.69 Å. The diffraction trace of a powder syn PHB octamer provided well-resolved *d* spacing measurements from which an orthorhombic unit cell of dimensions $a = 7.84$ Å, $b = 14.90$ Å, $c = 7.69$ Å is proposed. Conformational analysis with a syndiotactic diad repeat unit gave six low-energy chains with a fiber repeat of 7.69 Å. The most favorable chain conformation had dihedral angles similar to isotactic PHB but with a more extended integral helix. There is no energy preference for a right-handed or left-handed helix. The $P2_12_12_1$ space group was selected based on observed systematic absences, the tentative orthorhombic unit cell, and favorable packing energies. The 2_1 symmetry in the chain direction is between neighboring sets of anticline packed helices of syn PHB. The structural model of syn PHB put forth is based on favorable packing energies and a reasonable fit of simulated X-ray diffraction data with the experimental results, which were further evaluated by a Rietveld analysis.

Introduction

Poly(β-hydroxybutyrate), PHB, is a crystalline biopolyester made by various microorganisms as an intracellular carbon and energy reserve.[1−3] Once isolated from the microorganism, the polymer is a crystalline thermoplastic material similar in properties to polypropylene, but with the added advantage of being completely biodegradable. This biodegradability gives PHB and other members of the poly(β-hydroxyalkanoate) family many potential uses as an environmentally friendly thermoplastic. Furthermore, its biocompatibility enables uses as an orthopedic implant or surgical suture, in drug delivery, biodegradable packaging, and many other possibilities.[4]

Because natural PHB contains asymmetric centers with only the *R* configuration, this material is optically active and has a perfectly isotactic structure. The crystal structure of this polymer has been examined by X-ray diffraction[5−8] and found to take the form of a left-handed helix with a fiber repeat of 5.96 Å and a 2_1 screw symmetry along the chain axis. The $P2_12_12_1$ space group is in keeping with a minimum energy packing of antiparallel chains that readily form thin lamellar single crystals by chain-folding.[9]

Optically active isotactic PHB can also be made synthetically, by using either optically active monomer or stereoselective catalysts with racemic monomer. Ring-opening polymerization of racemic β-butyrolactone yields crystalline racemic PHB, the crystalline structure of which is identical by X-ray diffraction to that of fully isotactic polymer isolated from bacteria.[10−12] The crystal structure of the isotactic racemic polymer has been proposed to consist of two kinds of crystallites: one composed only of left-handed helices of the *R* chain segments, and the other composed of right-handed helices of the *S* chain segments.[7]

Recently, synthetic syndiotactic PHB, syn PHB, was found as a fraction when aluminoxane catalysts similar to those for the isotactic polymer were used.[13] Use of a stannoxane catalyst, which gives a preferentially syndiotactic placement with the racemic monomer,[14−17] led to preparation of a high-molecular-weight syn PHB. Although this material cannot yet be prepared to the same high levels of stereoregularity obtained for synthetic isotactic PHB polymers (69% syndiotactic diads versus 85−90% isotactic diads), it is sufficiently crystalline that its wide-angle X-ray diffraction powder pattern was distinctly different from that of isotactic PHB. The crystalline behavior of this material at various levels of stereoregularity (59−71% syndiotactic diads) has been investigated,[18] but only preliminary findings on the crystal structure of this new material have been reported.[19]

In this study we investigated the crystal structure of syn PHB, using X-ray fiber and powder diffraction in combination with molecular modeling to establish its conformation and chain-packing.

Experimental Section

Polymer Origins and Characterization. A transparent syn PHB film comprising 69% syndiotactic diads having averaged molecular weights $M_n = 88\,000$, $M_w = 133\,000$ was used for X-ray fiber diffraction. The polymer film was cast from chloroform and slowly cold-drawn up to 3200% over several days. Thermal properties were measured on the "as received" chloroform-cast film by differential scanning calorimetry, calibrated with indium and isodecane, at a heating rate of 20 °C/min. Melting temperatures and enthalpies of fusion were measured from the first scan; glass transition temperatures (T_g) were measured from a second scan after quenching at 200 °C/min.

A small quantity (50 mg) of crystalline syn PHB octamer (*RSRSRSRS*) synthesized in the laboratory of Dieter Seebach of ETH, Zurich, was kindly provided to us, and an X-ray powder diffractometer trace was recorded to supplement the X-ray fiber diffraction data. A combination of analytical data collected by ^{1}H and ^{13}C NMR, Fourier transform IR, and mass

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spectrometry confirmed the integrity of the chemical structure as well as the purity of the synthesized sample.[20] The octamer possesses a measured molecular weight of 707.2 (theoretical: 706.7) and a melting temperature of 118−118.5 °C.

X-ray Diffraction. The cold-stretching method for orientation used with the 69% syn PHB film as well as the X-ray fiber diffraction setup has been adequately described in a previous publication.[19] To conclusively prove the presence or absence of reflections along the meridional direction, we mounted the oriented fiber under slight tension on a tilting fiber holder, which could be adjusted to tilt the fiber axis up to 30° from vertical. Because a meridional reflection was observed for an apparent second layer line in the untilted fiber diagram at $2\theta = 23.4°$, exposures were made at 6°, 12°, 18°, and 24°. These confirmed the 002 indexing for this reflection, whereas the 001 reflection was absent.

An X-ray powder diffractogram of the syn PHB octamer was recorded on a Siemens X-ray diffractometer operating at 40 kV and 30 mA with graphite-filtered CuKα radiation. The one-dimensional (1D) powder scan was carried out in the $\theta/2\theta$ reflection mode in increments of 0.03° with a step time of 8.0 s. The finely ground octamer powder was adhered to the glass sample holder by means of a thin film of silicone grease that, in itself, added only a weak amorphous halo centered at $2\theta = 23.5°$ to the resulting diffraction pattern.

Density Measurement. Densities of a section of oriented and annealed fiber and of the syn PHB octamer were measured by flotation/titration. A stock sodium bromide solution was prepared and was titrated with water until a bead of standard density (1.2500 or 1.3300 g/mL, purchased from Ace Glass Co., Vineland, NJ) sank into the body of the solution and remained suspended. The concentration of the stock solution was then determined by using a least-squares fit of density versus concentration for sodium bromide solutions as tabulated in the *CRC Handbook of Chemistry and Physics*.[21] For titration of the polymer fiber, vigorous shaking of the titration vessel after each addition of titrant was required to overcome surface tension effects. The "as received" octamer flakes were best titrated in an organic solvent−based system of carbon tetrachloride and ethanol, which was calibrated with the 1.2000, 1.2500, and 1.3300 standard density beads.

Modeling Techniques. All molecular modeling was done with Cerius^2 (version 2.0), a program developed by Molecular Simulations Inc. A syndiotactic dimer was adopted as the asymmetric unit. Six torsion angles were systematically varied in 120° steps (60°, 180°, 300°), keeping the planar trans arrangement for the ester group; this generated 729 conformers. All these conformers were transformed into a regular "infinite" integral helical polymer and minimized to relieve unfavorable interactions. Bonds, angles, and the pitch of each polymer conformer were minimized by using the conjugate gradient and the Universal 1.01 force field.[22] The best-minimized chains were then packed into a reasonable crystal structure by using the crystal packer module of Cerius^2 based on the Dreiding II force field.[23]

The unit cell of the syn PHB octamer was derived from powder diffraction data by using the DICVOL91 powder indexing algorithm.[24] To facilitate the unit cell search, we restrained the lengths of crystallographic axes *a* and *b* to <20 Å and did not allow the *c* axis length to exceed 8 Å (the fiber repeat is 7.69 Å). Twenty to 24 reflections were used from the experimental octamer data for processing, and the experimental error of the Bragg reflections was varied from 0.1° up to 0.15°. The program allows for complete indexation (if a solution is feasible), an error analysis based on Q factors, and standard deviations for the unit cell parameters.

The best trial syn PHB crystal structure was subjected to a Rietveld (DBWS) or least-squares whole-pattern-fitting-structure refinement,[25] which attempts to improve the proposed crystal structure so that the simulated diffraction pattern becomes progressively more closely equivalent to the experimental one. For the syn PHB polymer fitted to the octamer 1D powder data, only the background and model variables were refined, with the gradual progress of best fit being monitored by the various agreement or R factors. Variables were refined to obtain constant values in 10 cyclical intervals having a tolerance factor of 0.3 before proceeding to the next level of refinement.

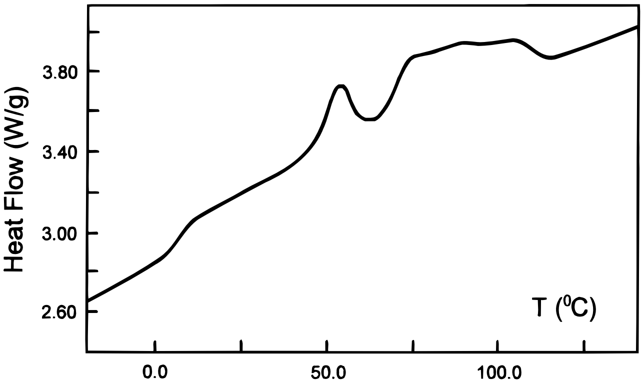


Figure 1. DSC thermogram of syn PHB film, as received. First heating scan.

Results and Discussion

Thermal Characterization of the 69% Syn PHB Sample. The DSC thermogram of the "as received" 69% syndiotactic film is shown in Figure 1. The observed T_g of 7.9 °C is slightly higher than the reported 1−5 °C range for syndiotactic PHB with 61−68% syndiotactic diads,[13] but within the −2.4 to 9 °C range reported for 59−71% syndiotactic diads.[18] All these values are near the −5 to +5 °C range reported for isotactic bacterial PHB.[26] As observed for other syndiotactic samples, several melting endotherms were observed over a broad temperature range.[18] The lowest melting point, 53 °C, is associated with a heat of fusion (ΔH_f) of 6 J/mol, whereas the broad endotherm composed of peaks from 76 to 105 °C has a ΔH_f of 24 J/g; the total ΔH_f is thus comparable with the values of 25−40 J/g reported for syn PHB with 64−71% syndiotactic diads crystallized from the melt.[18] The melting range of the current sample is also comparable with those previously reported for melt-crystallized samples.[18]

X-ray Fiber Diffraction Data. The X-ray diffraction pattern data for the oriented 69% syn PHB fiber has been quantified in terms of the average *d* spacing and intensity observed for each reflection (Figure 2 and Table 1). From the five different layer lines of the fiber diagram, up to 23 diffraction spots could be discerned. The most intense reflections were situated at *d* spacings of 5.50, 3.89, 4.48, 3.85, and 3.15 Å. Of most importance is the meridional reflection of the second layer line ($d = 3.85$ Å), which provides a fiber repeat of 7.69 Å for the polymer.

No evidence of (001) or (003) reflections was observed from the tilted diagrams. However, the (002) reflection was strongly enhanced, and a weak (004) reflection was recorded. The absence of odd-order reflections along the meridian suggests the presence of a 2_1 axis in the *c* direction of the unit cell. Although the *RS* backbone of syn PHB prevents the existence of a 2_1 axis along the chain, a 2_1 axis could exist between polymer chains.

Since an all-trans, fully extended PHB chain with a two-residue asymmetric unit (the minimum possible repeat for a syndiotactic arrangement) would measure ~9.6 Å, the syndiotactic crystal with a fiber repeat of 7.69 Å clearly does not involve the fully extended chain. However, the fiber repeat of the syndiotactic crystal is significantly larger than the 5.96 Å pitch of the isotactic twofold PHB helix, indicating that any hypothesized

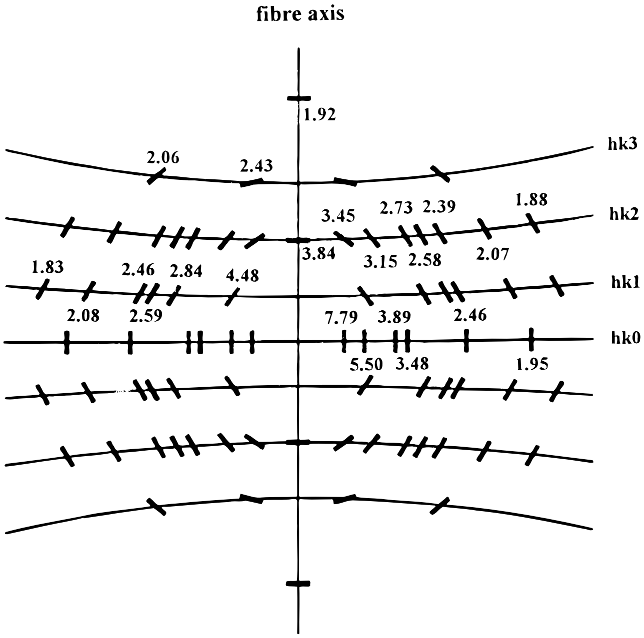


Figure 2. Schematic fiber diagram of all observed reflections, indexed as in Table 1.

Table 1. *d* Spacings (Å) Followed by Reflection Intensities[a] (in Parentheses) Summarized for the Five Distinct Layer Lines of the 69% Syn PHB Fiber Diagram

$l = 0$	$l = 1$	$l = 2$	$l = 3$	$l = 4$
7.79 (65)	4.48 (250)	3.85 (320)[c]	2.43 (23)	1.92[b] (12)
5.50 (383)	2.84 (39)	3.45 (44)	2.06[b] (12)	
3.89 (267)	2.59 (23)	3.15 (267)		
3.48 (95)	2.46 (23)	2.73 (43)		
2.46[b] (12)	2.08[b] (12)	2.58 (23)		
1.95[b] (12)	1.83[b] (12)	2.39 (23)		
		2.07[b] (12)		
		1.88[b] (12)		

[a] Quantified by aid of a NIH−image processing program applied to the digitalized fiber diagram. [b] Weak reflection measured on one film only. [c] Intensity derived from tilted fiber diagram.

syndiotactic helix must have a more-extended structure than the isotactic helix.

Because the stereoregularity is imperfect in this highly oriented fiber, crystalline disorder is probable in this highly cold-stretched (3200%) syn PHB sample. This disorder could arise from a large number of conformers with equivalent internal energy, from crystal imperfections, and from occasional isotactic diads (isomorphism) included within the syndiotactic crystallites. Given these concerns, only the pitch of the repeating unit derived from the fiber diagram has been retained for subsequent crystallographic analysis.

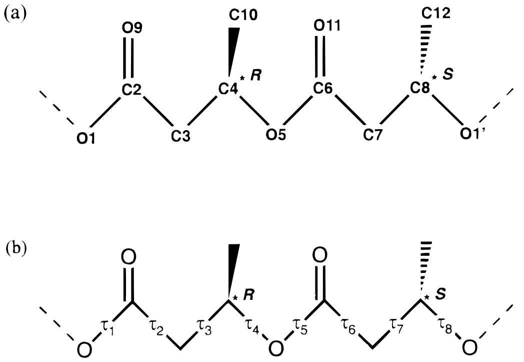


Figure 3. Schematic representation of syn PHB in a planar trans arrangement with (a) the numbering of the atoms and (b) the numbering of torsion angles.

Table 2. Energy (kcal/mol) of the Conformers with a Fixed Pitch of 7.69 Å

conformer	bonding	VdW	electrostatic	total
I	9.69	11.69	−148.0	−126.6
II	10.61	11.89	−152.5	−130.0
III	13.57	13.57	−153.5	−126.6
IV	10.81	11.09	−154.7	−132.8
V	14.59	12.01	−160.7	−134.1
VI	11.93	12.67	−152.1	−127.5

Modeling of the Syndiotactic Helix. The schematic representation of syn PHB in a planar trans arrangement with atoms and torsion angles numbering is shown in Figure 3. Systematic variation of torsion angles τ_2, τ_3, τ_4, τ_6, τ_7, and τ_8 gave 729 conformer chains. Twelve of these chains had an acceptable energy and a pitch close to the observed X-ray value of 7.69 Å. Half of the 12 chains were identical except for helix chirality. The six independent chains, designated models I through VI, are shown in Figure 4. The backbones of the remaining six chains are very similar except for the local chirality (*R* versus *S*) of carbons C4 and C8. When this chirality was changed randomly for any given chain, the internal energy of that conformation did not change significantly, implying that a low level of syndiotacticity does not influence the minimum energy of the polymer backbone. Table 2 summarizes the energy characteristics of these six different chains. Because the total intramolecular energies for these six different chains are similar, the ideal conformation cannot be selected based solely on the free-chain energy. Thus, crystalline packing considerations must be included in the investigation.

X-ray Powder Diffraction of Syn PHB Octamer. A reliable determination of the unit cell dimensions from the X-ray fiber diagram of the 69% syn PHB was not possible, in part because of the lack of stereoregularity of the polyester. In addition, given the fact that a β form of isotactic PHB is generated by cold-stretching,[27] the observed syn PHB fiber diagram may correspond to superimposed diffraction patterns of α and β crystalline forms. To eliminate these effects and to aid in the structure determination of the syn PHB polymer, we recorded a powder X-ray diffractogram (Figure 5) of the pure octamer *RSRSRSRS*. The 1D powder scan presents four major diffraction peaks at $d = 7.39$, 5.40, 4.40, and 3.74 Å and numerous weak, yet well-defined reflections at higher Bragg angles. The aforementioned peaks are present on the 69% syn PHB fiber diagram and have been reported in other works on 60−70% syn PHB.[13,14,18,28] A small but identifiable shoulder is present at approximately $d = 15.8$ Å, which, assuming it corresponds to a (002) reflection unique to the octamer crystallites, would correspond to an octamer length of 31.6 Å. Modeling any one of the six conformers as a true octamer gives an overall length between the protonated oxygen of the acid and the oxygen of the alcohol end group of 31.4 Å, which is in good agreement with the observed value. The base-plane dimensions of the isotactic PHB octamer are very similar[9] to those of the isotactic polymer, suggesting that the well-resolved syn PHB octamer diffractogram can acceptably represent the hypothetical syn PHB polymer. Comparing the *d* spacings of the octamer in Table 3 with those of the cold-stretched 69% syn PHB in Figure 2, we note that all major *d* spacings are common to the two sets of

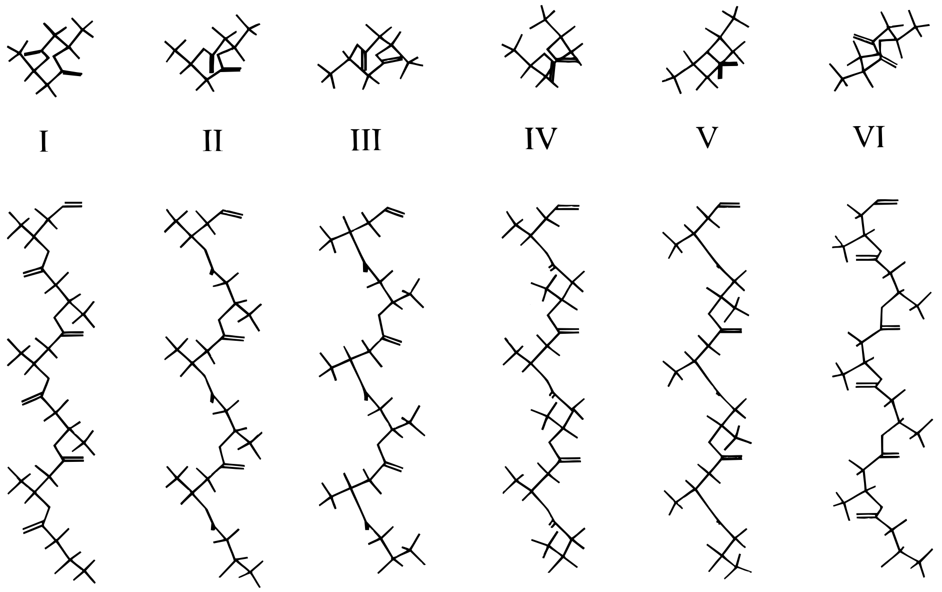


Figure 4. Representation of the six models with plausible internal rotation angles and a pitch of 7.69 Å.

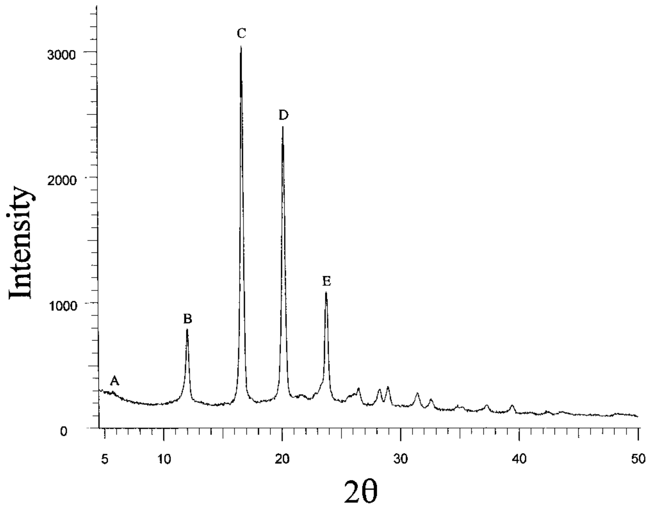


Figure 5. X-ray powder diffraction trace of "as received" syn PHB octamer (scan $2\theta/\theta$, step = 0.03°, step time = 8.0 s). Peaks A through E correspond to *d* spacings of 15.8, 7.39, 5.40, 4.40, and 3.74 Å, respectively.

data. We can thus conclude absence of the Beta phase in the 69% syn PHB fiber diagram.

Unit Cell Determination. The octamer powder diffraction data were used to determine the parameters of the unit cell by use of the following conditions. Only unit cells possessing a fiber repeat (*c* axis length) of 7.7 ± 0.1 Å were retained. Layer line *d* spacings from the fiber diagram were also taken into consideration, especially for the most intense reflections. Three plausible orthorhombic unit cells were generated that respect the condition of the fiber repeat (1: $a = 8.63$, $b = 13.69$, $c = 7.60$; 2: $a = 7.89$, $b = 14.64$, $c = 7.76$; 3: $a = 7.84$, $b = 14.92$, $c = 7.69$); however, only the third unit cell respects the major layer line conditions extracted from the 69% syn PHB fiber diagram. The plane indexation as well as the observed and calculated *d* spacings have been tabulated by using 23 reflections from the octamer powder diffractogram (Table 3). Measured *d* spacings of other, less stereoregular, syn PHB polymer samples have been included for comparison with the octamer data. Their values (*d* spacings and intensities) agree well with the octamer diffractogram trace, signifying the viability of applying data from the latter to represent the syn PHB polymer. In addition, the wealth of reflection information from the octamer provides confidence in the indexation of the unit cell.

The observed experimental densities measured by flotation on the 69% syn PHB film/fiber and on the octamer sample are 1.17−1.18 and 1.22 g/cm^3, respectively. An orthorhombic unit cell containing eight hydroxybutyrate residues ($Z = 4$) would give a predicted density of ~1.26 g/cm^3, which is in reasonable agreement with the experimental densities.

Crystalline Packing of the Conformers. Given the orthorhombic unit cell configuration and the absence of odd-ordered ($00l$) reflections along the meridional direction, the $P2_12_12_1$ space group was a logical choice for the packing of the syn PHB conformers in the crystalline lattice. In such a case, it is assumed that ($h00$) and ($0k0$) odd reflections are also absent, which is in line with the unit cell determination in Table 3. As the conformers themselves do not possess a 2_1 symmetry along the chain axis, this symmetry condition is required between the two sets of polymer chains that make up the orthorhombic unit cell. Overall symmetry requirements stipulate the packing of either right-handed or left-handed helices in an antiparallel arrangement. For convenience, we used the syn PHB left-handed helix in the crystal packing determination.

Crystal packing of each of the six conformers (I through VI) was carried out methodically by rotating (in 20° increments around the *z* axis) and performing *z* axis translations (from 0 to 3.8 Å in steps of 0.5 Å) of the asymmetric unit, after which the crystal packing of the resulting structure was minimized. Of all the conformers tried in this packing scheme, conformer I gave the crystalline packing arrangement with the best total energy of − 58.99 kcal/mol (VdW = −13.98, C. E. = −45.0). The packed conformers II through V in general gave positive energies because of the high VdW interactions, indicating their unsuitability for packing within the present unit cell. Reasonable crystalline packing was noted for model VI (total energy = −57.53 kcal/mol, VdW = −3.78, C. E. = −53.75); however, its packing is not as good as that of conformer I, primarily because of unfavorable VdW interactions.

Table 3. Unit Cell Parameters of Syn PHB Determined from 23 Reflections Measured from the Octamer Powder Diffraction Data[a]

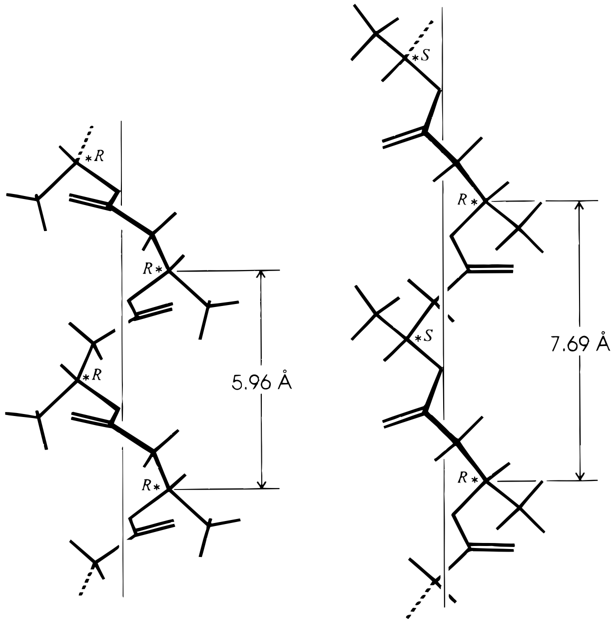
hkl	*d* observed, Å[b] octamer	69% film[28]	70%[14]	62–71%[18]	61–68%[13]	*d* calculated, Å
020	7.39s[c]	7.55m	7.52s	7.70s	7.66	7.45
120	5.40s	5.36s	5.40s	5.37s	5.49m	5.40
121	4.40s	4.43s	4.42s	4.44s	4.46s	4.42
200	3.91					3.92
002	3.83					3.85
210	3.81m	3.84s	3.83m	3.82m	3.85m	3.79
012 and 040	3.74m					3.73–3.72
131	3.69					3.68
220	3.47			3.48		3.47
022	3.42	3.42	3.44			3.42
112 and 140	3.37					3.37–3.36
221	3.16					3.16
230 and 141	3.09	3.09	3.11	3.14		3.08
231 and 132	2.85	2.86				2.86–2.84
202	2.75					2.75
222 and 310	2.58	2.56				2.58–2.57
241	2.55					2.55
113 and 232	2.41	2.35	2.35			2.41
033	2.28					2.28
331 and 242	2.20					2.21
312	2.13					2.13
322	2.08	2.07	2.06			2.08
153 and 411	1.88					1.89–1.88

[a] a = 7.84 Å, $\sigma = 0.013$; b = 14.90 Å, $\sigma = 0.023$; c = 7.69 Å, $\sigma = 0.011$; $\alpha = \beta = \gamma = 90°$, $\langle Q \rangle = 3.93 \times 10^{-2}$, $\Delta 2\theta = 0.0612$. [b] Known literature values of *d* spacings for syn PHB of various levels of tacticity % are included for comparison. [c] Qualitative reflection intensities: s = strong, m = medium (weak are not tagged).

Table 4. Bond Lengths and Bond Angles for Syn PHB (Conformer I)

bond	bond length (Å)	bond angle	angle measure, deg
O1–C2	1.406	O1–C2–C3	119.2
C2–C3	1.489	C2–C3–C4	112.6
C3–C4	1.532	C3–C4–O5	108.5
C4–O5	1.429	C4–O5–C6	117.6
O5–C6	1.408	O5–C6–C7	119.6
C6–C7	1.492	C6–C7–C8	114.0
C7–C8	1.533	C7–C8–O1′	109.1
C8–O1′	1.430	C8–O1′–C2′	116.6
O9–C2	1.221	O9–C2–C3	119.5
		O9–C2–O1	121.2
C10–C4	1.529	C10–C4–C3	108.6
		C10–C4–O5	109.1
O11–C6	1.220	O11–C6–C7	119.0
		O11–C6–O5	121.2
C12–C8	1.529	C12–C8–C7	111.8
		C12–C8–O1′	109.5

Conformer I was therefore selected as the chain conformation of syn PHB. Its bond lengths and valence angles are listed in Table 4. This conformer can also be obtained directly from the isotactic PHB chain by changing alternate asymmetric centers to the *S* configuration and then minimizing the energy to eliminate unfavorable interactions. This minimization involves allowing bond distances and angles to change and relaxing the requirement of a 2_1 axis along the chain backbone, in keeping with the observed fiber repeat of 7.69 Å (Figure 6). Comparison of the torsion angles of isotactic and syndiotactic chains in Table 5 indicates that the unfavorable *S*-methyl/carbonyl interactions are relieved primarily by changes in torsion angles τ_2 and τ_8 and also torsion angles τ_4 and τ_6, the angles most influenced in changing to the syndiotactic helix. Because the syndiotactic chain includes alternating *R* and *S* centers, there is no energy difference between left-handed and right-handed helices; we confirmed this by modeling both helices and determining their energies. In contrast, the dominant configuration in an isotactic sequence determines the handedness of the crystalline helix: *R*-PHB yields a left-handed helix, whereas *S*-PHB is a right-handed helix.[7]



(a) (b)

Figure 6. Comparison of the minimized helix of (a) isotactic and (b) syndiotactic model I PHB.

Simulated X-ray Powder Diffractograms. In Figure 7, a powder diffractogram was simulated on the best crystal packing of model I (curve b) and compared directly with the experimental octamer data (curve a). Such a crystalline packing gives reasonable simulated diffraction data, compared with the experimental curve. The intensities of the first three major reflections (020), (120), and (121) agree well with the corresponding octamer experimental data. The fourth major diffraction peak is underestimated by a series of three weak

Table 5. Torsion Angle Comparison for Isotactic and Syn PHB Conformer I

torsion angle	τ	isotactic	syndiotactic	difference
C8′−O1−C2−C3	1	−175, T[a]	-168.9, T	7.1
O1−C2−C3−C4	2	−47, −G	−96.2, −E	49.2
C2−C3−C4−O5	3	−52, −G	−63.1, −G	11.1
C3−C4−O5−C6	4	152, T	168.7, T	16.7
C4−O5−C6−C7	5	−175, T	−172.2, T	2.8
O5−C6−C7−C8	6	−47, −G	−67.2, −G	20.2
C6−C7−C8−O1′	7	−52, −G	−58.5, −G	6.5
C7−C8−O1′−C2′	8	152, T	−160.5, T	47.5

[a] Angles also represented by standard conformational notation T = trans, G = gauche, and E = eclipsed.

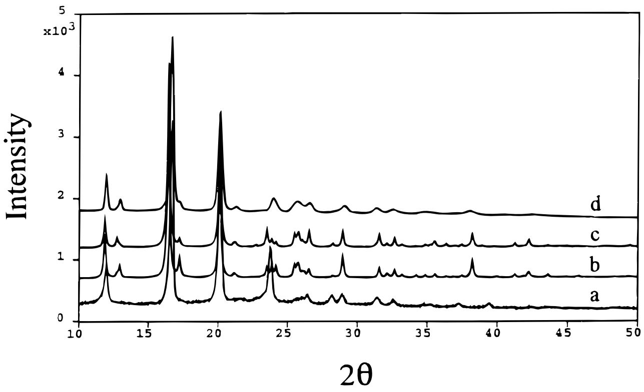


Figure 7. Series of X-ray powder diffractograms: curve a, experimental syn PHB octamer data; curves b and c, simulated diffraction patterns for models I and Ia, respectively, using a Lorentzian peak shape, (500 Å)^3 crystallites and B = 10 Å^2; curve d, Rietveld refinement applied to model Ia with parameters presented in Table 7.

Table 6. Observed and Calculated Structure Factors for Model Ia

plane	Fo (*hkl*)	Fc (*hkl*)
020	39.98	28.03
120	76.76	76.44
121	52.86	68.72
200	9.00	8.59
002	19.30	6.37
210, 040, 210	55.89	29.43
131	8.09	12.71
220	9.69	15.70
022	11.97	13.21
112, 140	15.60	24.86
221	13.59	12.23
230, 141	18.58	26.44
231, 132	14.50	26.29
202	16.88	21.26
222, 310	9.60	11.56
241	5.93	9.45
113, 322	9.76	13.15
033	17.01	0.66
331, 242	6.58	5.55
312	21.43	21.75
322	6.90	12.20
153, 411	11.81	7.15

reflections, attributable to (210), (040), and (012). A disagreement index or R Bragg factor was calculated according to the equation: $R = \sum||F_o| - |F_c||/\sum|F_o|$ and by including lorentz, polarization, multiplicity, and temperature (B) factors in the calculation. With respect to the 23 observed diffraction peaks, a R value of 35.3 has been evaluated by using B = 1 Å^2.

To achieve a better crystalline fit, the two methyl groups of the asymmetric unit were slightly rotated along their C−CH$_3$ rotational axes, thereby alleviating unfavorable atom−atom contacts between adjacent chains. Further energy minimization caused the crystalline energy to plummet to −120.1 kcal/mol (VdW = −42.32 and C. E. = −77.81). A similar attempt with model VI, using methyl rotation, did not produce better energies or an improved diffraction simulation. The crystalline packing energy of syn PHB is comparable with that calculated for the accepted crystalline structure of isotactic PHB (total energy = −76.5 kcal/mol, VdW = −29.1, and C. E. = −47.4). The modified crystalline packing of model I, model Ia (Table 6), improves the general appearance of the simulated diffraction pattern (curve c). Compared with the profile of curve b, the fourth major diffraction ($2\theta = 23.85°$) gains in intensity thanks to the (210) reflection. The unobserved diffraction peaks at $2\theta = 12.8{-}12.9°$, 17.2°, and 38.2° are appreciably weaker in intensity. A satisfactory disagreement factor of (R) 33.4 was calculated with use of this improved crystal structure for syn-PHB.

A Rietveld refinement of model Ia was attempted from Bragg angles of $2\theta = 10°$ to 50°. Refinement commenced with the background coefficients and unit cell parameters and proceeded, in order, with the peak profile fitting, orientation, and temperature factors before performing an overall refinement of all these variables combined at the end of the process. The final values for these refined variables are listed in Table 7 together with the calculated R factors particular to the refinement technique. Curve d on Figure 7 is the simulated diffractogram after Rietveld refinement of model Ia. An improvement in overall fit is observed going from curves c to d but at the cost of slightly modifying the earlier unit cell dimensions determined by the DICVOL 91 method. Glancing at Table 7, we note that the Reitveld refinement process has interchanged the crystallographic lengths of the a and c axes on account of their similarity in value and has decreased the *b* axis dimension from 14.90 to 14.81 Å. Despite these modifications, the unit cell density remains relatively constant. Free-chain modeling of syn PHB with the increased fiber repeat distance of 0.15 Å had little effect on the total energy of conformer I, thus suggesting the existence of a certain margin in its real value. The observed R factor of 32.65 calculated from 215 data points is acceptable for a preliminary model structure of 100% syn PHB polymer. However, during the whole peak-fitting process, it was necessary to introduce an orientation parameter and to use a higher than normal temperature factor to dampen overall simulated intensities at higher Bragg angles. An attempt to perform a slight coordinate refinement of atomic positions led to unrealistic changes in bond distances and angles as well as torsional angles. Such refinements of polymer systems that crystallize in the form of helices are possible by defining a generalized coordinate system.[29] Notwithstanding the obvious strengths of the Rietveld refinement method, we consider model Ia to be the most experimentally coherent with respect to the data available at this time for the syn PHB polymer. Model Ia should be considered an acceptable preliminary model of the syn PHB crystalline structure.

Structural Model for 100% Syn PHB Polymer. The best crystalline packing arrangement of syn PHB corresponding to model Ia is illustrated in Figure 8; its fractional coordinates are presented in Table 8. The a−b and c−b crystallographic projections of syn PHB polymer illustrate the close packing of four helical chains in an orthorhombic unit cell that respects the

Table 7. Rietveld Refinement Final Values for Model Ia

background coefficients: C1 = −142.1, C2 = −485.4, C3 = −66.28, C4 = −12.56, C5 = 44.69, C6 = 122.0
unit cell variables: a = 7.69, b = 14.81, c = 7.86
profile fitting: pseudo-Voigt U = 6.91, V = -0.888, W = 0.060, A = 0.5 (equal weighting of Lorentzian and Gaussian functions)
orientation parameter: March Dollarse, G1 = 1.0347 [001]
global temperature factor: 14.29
R-P = 16.26%, R-WP = 22.27%, R Bragg = 32.65%

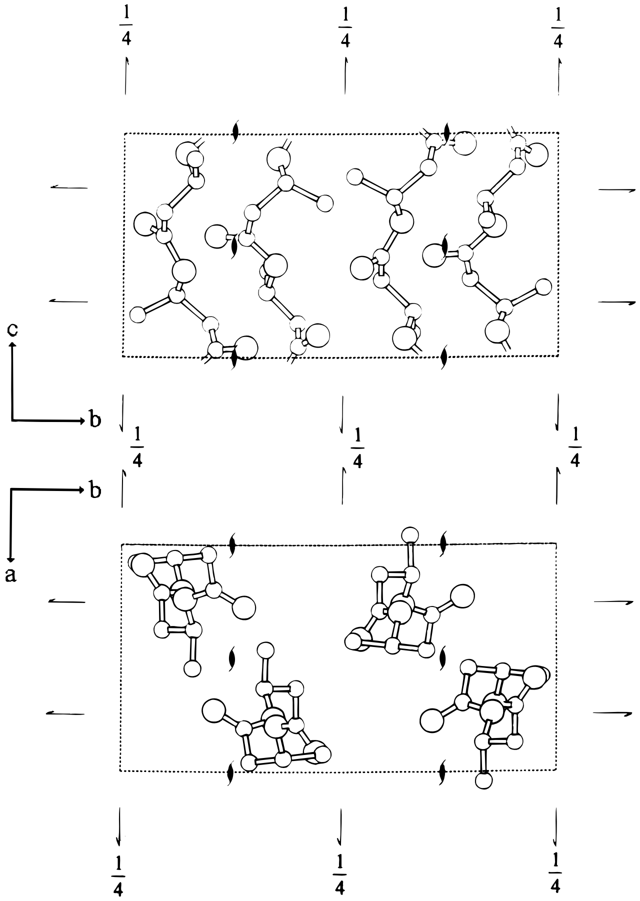


Figure 8. Crystal structure of syn PHB polymer based on model Ia. The small and large circles represent carbon and oxygen, respectively; the hydrogens have been omitted for increased overall clarity.

Table 8. Fractional Coordinates for the Asymmetric Unit of Syn PHB Polymer

atom	*x*	*y*	*z*
O(1)	0.2371	0.1484	0.9137
C(2)	0.1941	0.2148	0.03629
O(9)	0.2733	0.2851	0.04470
C(3)	0.04155	0.2020	0.1497
H(3a)	0.02099	0.2648	0.2240
H(3b)	−0.07110	0.1910	0.06518
C(4)	0.05946	0.1252	0.2807
H(4)	−0.06088	0.1248	0.3597
C(10)	0.07965	0.03454	0.1903
H(10a)	0.2055	0.00521	0.2240
H(10b)	−0.02242	−0.01274	0.2306
H(10c)	0.07464	0.04339	0.04726
O(5)	0.2045	0.1416	0.3889
C(6)	0.2104	0.09529	0.5480
O(11)	0.08940	0.05074	0.5983
C(7)	0.3711	0.09624	0.6513
H(7a)	0.3866	0.02942	0.7123
H(7b)	0.4786	0.1076	0.5600
C(8)	0.3706	0.1684	0.7931
H(8)	0.3504	0.2359	0.7319
C(12)	0.5420	0.1657	0.8879
H(12a)	0.6049	0.2321	0.8810
H(12b)	0.6292	0.1161	0.8272
H(12c)	0.5218	0.1483	0.02662

overall $P2_12_12_1$ symmetry. Because 2_1 symmetry is not possible along the helical chain axis for syn PHB, it is accounted for between pairs of chains. van der Waals interactions are minimized in such a packing arrangement, the closest unfavorable contact between the methyl and the ester chain oxygen of the first set of chains occurring at ~2.4 Å. Stability between adjacent chains seems to come from the alignment of the ester groups within the same plane, which accounts for the favorable coulombic energy interactions in the packing of the helical chains. The crystalline packing as illustrated assumes a left-handed helix for the polymer chain. An all-right-handed helical conformer generated from model Ia and its crystalline packing have been determined and give the same overall packing energy and simulated diffractogram as the left-handed helical model.

Conclusions

The crystal structure of syn PHB was examined by using X-ray diffraction techniques and molecular modeling. A fiber repeat of 7.69 Å per 2 repeat units was measured from the fiber diagram of a highly cold-stretched sample of 69% syn PHB. A pseudo twofold helical conformation similar to that of isotactic PHB was proposed but with an extended advance per chemical repeat unit, which caused significant changes in some of the torsion angles. Refinement calculations of the unit cell, based on the well-resolved syn PHB octamer reflections (which also correspond to the 69% syn PHB fiber diagram data and systematic absences), suggest the crystallization of the pseudo-helix in an orthorhombic unit cell (a = 7.84 Å, b = 14.91 Å, c = 7.69 Å) respecting the symmetry requirements of the $P2_12_12_1$ space group.

Recent efforts have been put forth to grow PHB octamer single crystals from dilute solution for subsequent electron diffraction analysis. Preliminary results have shown that the octamer can be crystallized to form lath-like single crystals. Diffraction patterns on such structures would verify the base-plane parameters proposed for the syn PHB polymer.

The 69% syn PHB is probably biodegradable because of the inclusion of *R* stereoblock sequences along the chain backbone.[30] The low level of syn PHB stereoregularity provides certain desired physical characteristics such as greater transparency and toughness than the 100% isotactic PHB. The 100% syn PHB polymer may also possess some of these desirable properties as well as a fusion temperature conducive to improved processability that would minimize overall chemical degradation.

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