

Molecular modelling of helical and extended-chain polyhydroxybutyrates and polytetramethylene succinate†

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Molecular modelling (MM) has been employed to investigate the crystalline chain conformations of a series of related aliphatic biodegradable polyesters. The bacterial (isotactic) poly(3-hydroxybutyrate) helical conformation of 2.98 Å advance/repeat unit can be cold-drawn to a nearly extended chain conformation possessing an advance of 4.6 Å/repeat unit *versus* 4.8 Å for complete extension. Syndiotactic PHB has an extended helical chain conformation of 3.85 Å per repeat on account of steric interference between substituents of contiguous R and S units. Molecular modelling techniques have confirmed this result and suggest that up to 25% of S configured units spread randomly or in a stereoblock fashion are isomorphous with the R repeats of isotactic helix segments. From X-ray fibre diffraction data, a low energy crystalline chain conformation for elastomeric poly(4-hydroxybutyrate), P4HB, with a pitch of 11.9 Å per 2 repeat units has been proposed. A synthetic biodegradable polyester, poly(tetramethylene succinate) or Bionolle®, undergoes a reversible strain-induced crystal modification in which the fibre identity period increases from 10.91 Å to 11.97 Å. Plausible conformers are proposed respecting these observed pitches. Modelled P4HB and Bionolle® chains in the 'all *trans*' planar zigzag form correspond to a pitch of 12.4 Å, which overestimates the observed fibre repeat values. Analysis of the crystalline structure of synthetic and bacterial polyesters gives potential insight into their biodegradability, which is controlled by specific enzyme binding characteristics. © 1998 Elsevier Science Ltd. All rights reserved.

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INTRODUCTION

X-ray fibre diffraction from highly oriented semi-crystalline polymers is routinely used as a tool to derive the minimum energy conformation and subsequent packing of the polymer chains within the crystal lattice. In the case of aliphatic polyesters, the method is valuable for comparing the effect of regular substitution in the repeating unit on the pitch and symmetry of linear chains. This is especially true for natural polymers such as poly(3-hydroxybutyrate), PHB, the most ubiquitous of the bacterial carbon reserve polyhydroxyalkanoates, which is the starting point of this investigation.

Lemoigne¹ recognized in 1925 that the microscopic refractile granules observed in bacteria were insoluble in ether and, hence, discarded the idea that they could have been lipids. Several analyses—optical rotation, saponification number, melting point and solubility variation with molecular weight etc.—brought him to propose a long chain structure based on R-3-hydroxybutyric acid as the repeat unit. This structure was proposed at a time when scepticism about the macromolecular hypothesis was still rampant. Apparently, Staudinger's hypothesis about the existence of

giant molecules was more accepted by organic chemists such as Carothers² than by physical chemists at that time.

What has changed in the polyhydroxyalkanoate field in the past decade or more is the discovery that a large number of different repeat units are found in these reserve materials, depending on the bacterial species and ingested substrate. This is the basis of the biosynthesis of the copolyesters, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)³, known as Biopol®, which constitute the family of available thermo-plastic material currently in development and use. Although not all bacteria make polyhydroxyalkanoates (PHA) as a carbon reserve, the material is so widespread among bacteria that they are now well accepted as a separate class of biopolymers⁴. Some 91 different repeat units have now been identified from natural sources⁵.

In addition, most poly(3-hydroxyalkanoate)s possess an R configured carbon in the backbone which means that bacterial PHB is chiral and 100% isotactic. When the chemical synthesis of PHB was first reported using racemic β-butyrolactone as monomer⁶, the X-ray diffraction pattern of a non-optically active high molecular weight product was found to be the same as that of the bacterial optically active material. Yokouchi *et al.* proposed that this is due to synthesis of a stereoblock product where blocks of *rectus* and *sinister* units crystallize separately. This hypothesis remains in use and the molecular chains from synthesis of a racemic monomer mixture has been shown by NMR analysis⁷ to follow Markovian laws of sequence distribution. However, it seems likely that isomorphous inclusion of

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† This paper is dedicated to our colleague, Dieter Seebach, on the occasion of his 60th birthday. His innovative chemistry and insightful analysis of poly(3-hydroxyalkanoate)s have brought enlightenment and high standards to the field of biodegradable polymers

Table 1 Summary of poly(hydroxyalkanoates) investigated by MM

Chemical formulae	Name and acronym
$\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}-\text{CH}_2-\text{C}-\text{O} \\ \\ \text{CH}_3 \end{array} \right]_n$	Poly(3-hydroxybutyrate) PHB
$\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}-\text{CH}_2-\text{C}-\text{O} \\ \\ \text{CH}_3 \end{array} \right]_x \left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}-\text{CH}_2-\text{C}-\text{O} \\ \\ \text{CH}_2\text{CH}_3 \end{array} \right]_y$	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHB/V
$\left[\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{C}-\text{O} \\ \\ \text{H} \end{array} \right]_n$	Poly(4-hydroxybutyrate) P4HB
$\left[\begin{array}{c} \text{O} \quad \text{O} \\ \parallel \quad \parallel \\ \text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}-\text{CH}_2-\text{CH}_2-\text{C}-\text{O} \\ \quad \\ \text{H} \quad \text{H} \end{array} \right]_n$	Poly(tetramethylene succinate) PTMS

some *sinister* repeat units in *rectus* crystallites is present in synthetic PHB which is not 100% isotactic. This situation is explored in this study along with a conformational examination of crystalline heterotactic and syndiotactic PHB.

Finally, this study dwells on the challenge of understanding the conformation of extended chain forms of both natural and synthetic PHA. Occasionally, PHAs such as poly(4-hydroxybutyrate) (P4HB) are found in nature as optically neutral polymers. Such materials seem to adopt a planar zigzag conformation on first analysis. A similar synthetic material of the non-polar chain type: poly(tetramethylenesuccinate), Bionolle[®], has been reported as a biodegradable fibre and plastic⁸.

Also reported as extended chains are cold-drawn versions of PHB and P(HB-co-HV) copolyesters. The crystalline conformations of these materials was studied using Cerius² as the modelling strategy along with some new X-ray fibre diffraction data. The molecular structure of these materials is conveniently summarized in Table 1.

EXPERIMENTAL

Materials and sample orientation

A transparent chloroform-cast syndiotactic PHB film (69% syndiotactic diads, $\bar{M}_n = 88\,000 \text{ g mol}^{-1}$ and $\bar{M}_w = 133\,000 \text{ g mol}^{-1}$) was supplied by Dr Hori of Takasago International Corporation⁹. The film was securely clamped in a manual turn-screw-style stretching device in which the sample length could be slowly increased to prevent film rupture. Alternating room temperature stretching and annealing under tension produced the best X-ray fibre diagrams. Initially, the film was stretched to 600–1000% and then annealed under tension at 83–85°C for 30 min. Removal of the fibre from the clamps after cooling to room temperature caused only a slight retraction of the fibre (80–90% of the length under tension). Weak orientation was observed by X-ray diffraction on such drawn fibres. Hence, the film was remounted and restretched to 1800–2400% and again annealed under tension at 84°C. A third stretching and annealing improved the fibre orientation and an X-ray diffraction diagram is reported for a sample drawn to 3600% of its initial length.

The elastomeric P4HB provided by Dr A. Steinbüchel was dissolved in chloroform and cast as a smooth film. It was subsequently heat-stretched under tension just below its melting temperature in order to generate a highly oriented fibre for X-ray diffraction.

X-ray fibre diffraction

The X-ray diffraction work was carried out using a Philips PW 1730 X-ray generator operating at 40 kV and 20 mA. A collimated X-ray beam (0.015 in.) of $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) was employed in series with an evacuated Warhus–Statton camera using flat film detection. NaF calibration was used for both polyesters. The sample-to-film distance was 5 and 3 cm for syn-PHB and P4HB, respectively. Film exposition times ranged from 17 h for syn-PHB to 24 h for P4HB.

Conformational analysis

All molecular modelling was carried out using Cerius² software (Molecular Simulations Inc.) on a Silicon Graphics workstation. Free chain conformations of the polyesters were determined by applying a Universal 1.01 force field which takes into account bonding (bond stretching, valence angle bending, torsional twisting, and improper or out of plane torsions) as well as non-bonding interactions: van der Waals' (vdW) forces, coulombic energy (CE) interactions in the energy minimization procedure. The charges of the various atoms were recalculated every 50 iterations by using a charge equilibration method (Qeq) which depends directly on the geometry of the conformation and the electronegativities of the constituent atoms. Energy minimization was carried out with the help of a conjugate gradient algorithm and it successfully terminated when the root-mean-square (RMS) force was less than $0.100 \text{ kcal mol}^{-1} \text{ \AA}^{-1}$. Single free chain minimizations were performed within a unit cell with dimensions of $a = 50 \text{ \AA}$, $b = 50 \text{ \AA}$, $c = \text{pitch of chain (}\text{\AA}\text{)}$.

The large a and b terms virtually eliminate interchain contributions to the total energy and the as-defined unit cell assures the continuity of the free chain along the c direction. Using the Minimizer module, high positive stresses applied along the chain direction caused it to extend to high absurd

energy values. The whole system was thereupon allowed to relax stress-free along the *c* direction to a minimum energy conformation. Other attempts to find suitable low energy conformations were achieved by using negative stress values along the chain direction which compressed the chains to low pitch values and high energies. The stress was then relieved to permit energy minimization of the conformer.

Crystal packing energies of the different crystalline PHB structures were achieved within a Dreiding II force field. In this case, only van der Waals' and coulombic interactions contribute to the overall packing energy between the chains of the polyesters. Energy minimization was performed by use of a modified Newton algorithm.

Torsional angle values for the preferred crystalline conformer states have been depicted using the following standard notation (*cis* = $C = 0^\circ$, + *gauche* = $G = 60^\circ$, - *gauche* = $\bar{G} = -60^\circ$, + eclipsed (or skewed) = $E = 120^\circ$, - eclipsed = $\bar{E} = -120^\circ$ and *trans* = $T = 180^\circ$).

RESULTS AND DISCUSSION

Poly(4-hydroxybutyrate)

The initial detailed structural determination of isotactic PHB using crystallographic methods has been undertaken over 30 years ago by two research groups^{6,10,11}. In both cases, a rigorous conformational analysis was performed followed by a conformer packing and minimization of the difference between observed and calculated X-ray structure factors. Yokouchi *et al.* undertook an extra refinement of the atomic positions and thermal coefficients by means of a diagonal least-squares method. In both studies, the PHB crystal lattice parameters were found to be:

- an orthorhombic unit cell where $a = 5.76 \text{ \AA}$, $b = 13.20 \text{ \AA}$ and c (fibre repeat) = 5.96 \AA ;
- and two chains per unit cell each possessing a twofold screw axis and packed in an anti-parallel arrangement ($P2_12_1$ space group).

Yokouchi *et al.* used both bacterial PHB (100% isotactic) and lower tacticity synthetic racemic polymer consisting of isotactic blocks where all the *rectus* and all the *sinister* blocks form left- and right-handed helices, respectively. They hypothesize that these stereoblocks crystallize in separate homogenous crystallites.

Brückner *et al.*¹² have refined the Yokouchi iso-PHB structure by using the Rietveld whole fitting method applied to X-ray diffraction data. They have proposed a modified PHB crystalline structure in which their orthorhombic unit cell consists of slightly altered crystallographic parameters ($a = 5.73 \text{ \AA}$, $b = 13.14 \text{ \AA}$ and $c = 5.93 \text{ \AA}$). However, they state that these results can be influenced by amorphous scattering and the presence of preferred orientation in the crystalline regions. As a consequence, it would be

informative to compare the reliability of the three proposed crystalline structures of iso-PHB by using Cerius² modelling techniques. Table 2 summarizes the calculated energies for the proposed crystalline structures of bacterial PHB.

Calculated reliability indices for the three models range from 0.34 to 0.15. All three models give similar energies for the left-handed 2_1 helix, with models A and C giving respectable free chain energy values. Although model A did not allow deviation from the planar-*trans* ester conformation, the Cornibert/Marchessault chain conformation possesses favourable bonding term interactions, lowering the total free chain energy. Model B seems to rely on a quite favourable internal coulombic interaction which compensates for the high vdW energy term. Model C is an improvement on model B, seen by the decrease in unfavourable vdW interactions.

Upon packing the free chains, it is evident that model C possesses a superior packing energy compared with the other two. Given the consistency of all three vdW values, the Brückner model relies on highly favourable interhelical coulombic interactions. Comparing the free chain and packing energies of models A and B confirms the fact that a conformer of less favourable energy can be packed to give a more favourable packing energy. Despite the fact they have similar reliability factors, model C seems to be a more sound choice over model B owing to its superior crystal packing energy. Molecular modelling has confirmed the degree of relative reliability of the three models and supports the viability of model C over the other two. As a direct consequence of these conclusions, the Brückner free chain model of the crystalline state has been used in the subsequent molecular modelling analysis. However, all three studies have converged to models whose major molecular and crystallographic characteristics are essentially the same, thus providing considerable confidence about the helical conformation, symmetry and handedness of the PHB helix.

Syndiotactic poly(3-hydroxybutyrate)

Knowledge of the crystalline organization in bacterial PHB has been applied to analyse a recently synthesized^{9,13,14} syndiotactic PHB. An eloquent crystallographic/molecular modelling analysis has recently been carried out on a 69% syndiotactic sample of PHB¹⁵. Preliminary results from the X-ray fibre diffraction pattern (Figure 1) suggests that it crystallizes in an orthorhombic unit cell with $a = 7.84 \text{ \AA}$, $b = 14.90 \text{ \AA}$ and $c = 7.69 \text{ \AA}$. Inverting one *rectus* carbon to the *sinister* form in the all R 2_1 helix crystalline conformation introduces an unfavourable contact between the β methyl and the carbonyl of the ester moiety. To alleviate this unfavourable high energy conformation, the 2_1 helix extends itself along the backbone direction, preserving the overall helical arrangement thanks

Table 2 Free-chain and packing energies from Cerius² modelling on the proposed crystalline structures of iso-PHB based on X-ray diffraction data. *R* is the crystallographic reliability factor

Model ^a	<i>R</i>	Free chain energies (kcal mol ⁻¹)				Packing energies (kcal mol ⁻¹)		
		Bonding	vdW	CE	Total <i>E</i>	vdW	CE	Total <i>E</i>
A	0.34	114.6	21.3	-124.0	11.9	-28.7	-13.5	-42.2
B	0.20	122.2	29.0	-133.5	17.7	-28.5	-14.7	-43.2
C	0.15	121.3	19.1	-129.9	10.5	-29.1	-47.4	-76.5

^a(Model A—Cornibert/Marchessault¹¹, model B—Yokouchi *et al.*⁶ and model C—Rietveld refinement method applied to model B (Brückner *et al.*¹²))

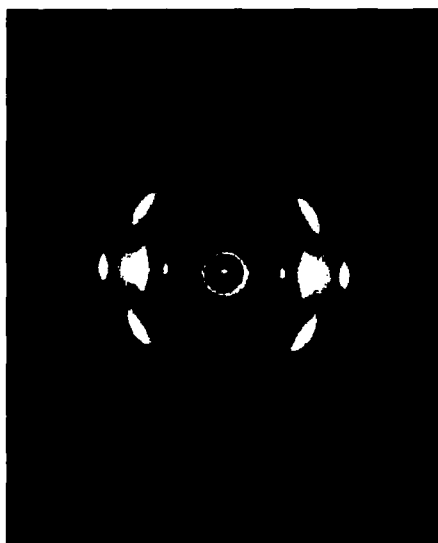


Figure 1 X-ray fibre diagram of oriented 69% syndiotactic PHB. The meridional reflections of the 2nd layer line provides a fibre repeat of 7.69 Å. The stretching direction is vertical and the highly oriented fibre was held perpendicular to the X-ray beam

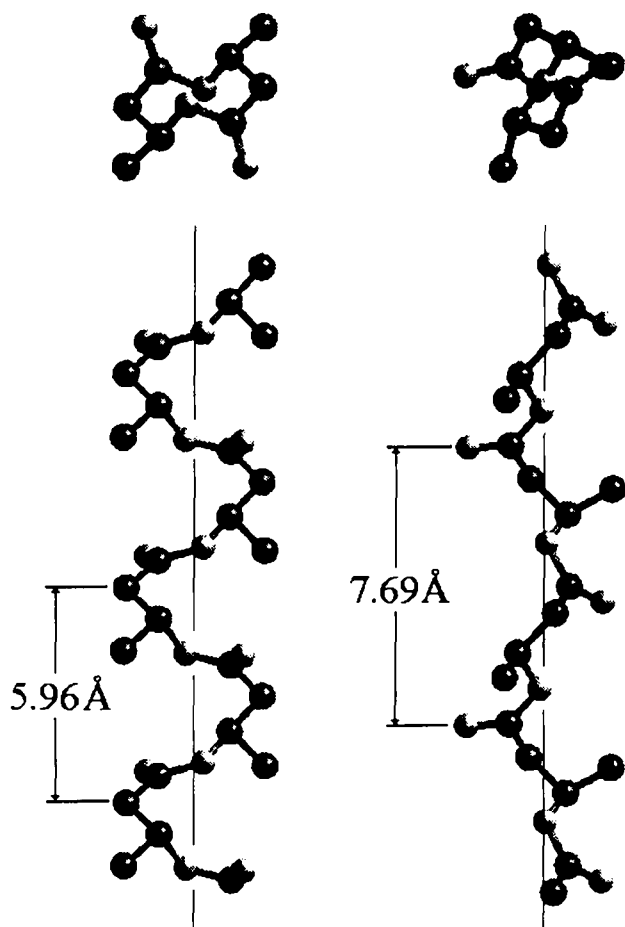


Figure 2 Orthogonal and down chain views of left-handed 2_1 isotactic PHB and extended-chain helical syndiotactic PHB

to internal intramolecular coulombic interactions (Figure 2). Since a 100% syndiotactic PHB has yet to be synthesized, an attempt was made to understand the chain conformation of imperfect stereoregular material by MM methodology. At this stage, however, we have confidence in the pitch of the syndiotactic structure (7.69 Å).

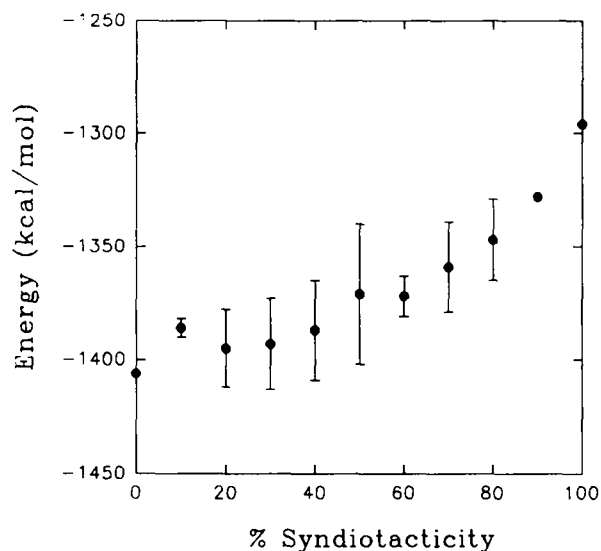


Figure 3 Graph depicting the progressive addition of S units on to an all R iso-PHB free chain of 10 dimers. Four S units per 10 dimers corresponds to 40% syndiotacticity. The filled circle represents the average free chain energy value and the error bars take into account Markovian and Bernoullian addition of S sequences

Commencing with an all R configured isotactic 10 dimer free chain of PHB, an increasing number of *rectus* carbons were systematically mirrored to *sinister* along the backbone, and minimized energywise while keeping the pitch constant for iso-PHB (59.6 Å/10 dimers). The energies of the resulting minimized free chains have been plotted in Figure 3 as a function of S units incorporated in the backbone. Bernoullian (random) and Markovian (stereoblock) type arrangements of the S units were considered in order to investigate all permutations of S units within the all R 10 dimer sequence. The minimized Bruckner free chain gives an energy of $-1410 \text{ kcal mol}^{-1}$ per 10 dimers. The stepwise addition of S has a general negative impact on the total energy for all combinations attempted. However, it is interesting to note that some sequence combinations give reasonable energies (less than -1400 kcal/mol) up to approximately 50% syndiotacticity. No particular preference was observed between random and stereoblock type free chains. Beyond 50% syndiotacticity, the total energy progressively worsens and it is evident that a 10 dimer 100% syndiotactic chain with a 59.6 Å pitch is unfavourable even when it is minimized. The fact that favourable energies are received by modelling up to 50% syndiotacticity suggests that S incorporation into an all R chain may be feasible up to these values of stereoregularity.

α-β transformation of cold-drawn poly(hydroxyalkanoates)

While syndiotactic PHB exists in an extended helicoidal conformation compared with isotactic PHB, cold drawing the latter leads to a unique extended-chain conformation. Orts *et al.*¹⁶ have demonstrated that bacterial poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHB/V, with 21% HV molar content, exhibits a thermally reversible α - β transformation from the helical conformation to a planar zigzag form, similar to a behaviour observed in steam-stretched wool. The cold-drawn form has an advance of 4.6 Å/repeat unit compared to 2.98 Å for the regular isotactic PHB helix. This result is in agreement with Yokouchi *et al.*, who measured a value of 4.7 Å/repeat unit for strained synthetic racemic PHB. The completely extended chain in

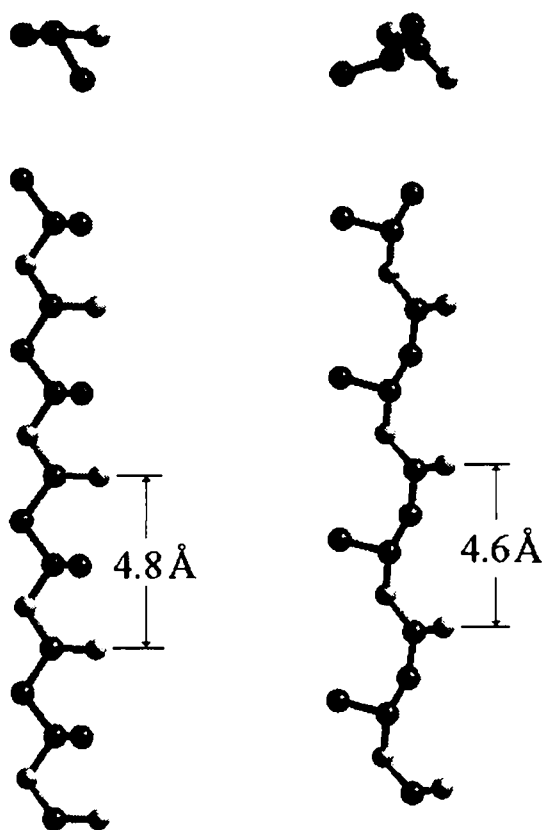


Figure 4 Orthogonal and down chain projections of modelled 'all trans' (4.8 Å) and cold-stretched extended-chain conformers of iso-PHB (4.6 Å)

Table 3 Advance/repeat and torsional angles for iso-PHB in the helicoidal (2_1), cold-drawn (nearly extended 2_1) and 'all trans' modelled forms

Repeat unit (Å)	$[-\text{CH}(\text{CH}_3)]$	$-\text{CH}_2$	$-\text{C}(\text{O})$	$-\text{O}-$	Form
2.98	T	\bar{G}	\bar{G}	T	α
4.6	E	T	\bar{E}	T	β
4.8	T	T	T	T	'all trans'

the 'all trans' conformation has been modelled and possesses a pitch of 4.8 Å, a value slightly larger than the cold-drawn sample. These results are depicted in *Figure 4* and are compared in *Table 3* where the torsional angles have also been tabulated in correlation with the advance repeat. First, the 'all trans' chain is not observed experimentally, most likely because the vdW radii of the methyl groups overlap those of the carbonyl oxygen. As a direct consequence, regular kinking takes place in two different positions along the backbone, with one directly adjacent to the carbonyl of the ester group ($\text{C}(\text{O})-\text{CH}_2$) and the second position along the $\text{O}-\text{CH}(\text{CH}_3)$ bond. The overall kinking pattern is repeatable through a translational symmetry operation which makes the cold-drawn β chain regular in its three-dimensional appearance. Sidechain aliphatic polyesters are known to kink preferentially at the $\text{C}(\text{O})-\text{CH}_2$ bond⁶.

This β form of iso-PHB is reminiscent of cold-drawn polypivalolactone¹⁷, which makes oriented fibres with remarkable short extension reversibility. This is attributed to a reversible α - β local transition in the fibres¹⁸. A similar property is probably to be found in oriented PHB fibres.

Poly(4-hydroxybutyrate)

Poly(4-hydroxybutyrate) is a biodegradable thermal

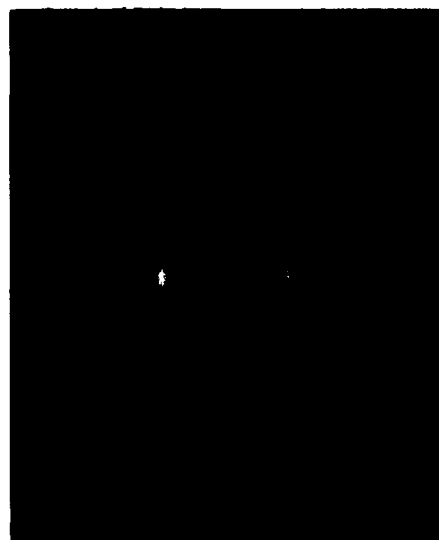


Figure 5 X-ray fibre diagram of heat-stretched P4HB. The intense meridional reflections of the 2nd layer line correspond to a fibre repeat of 11.9 Å

Table 4 Summary of pitches, torsional angles and corresponding free chain energies for the various P4HB conformers^a generated by Cerius² modelling

Pitch (Å)	$[-\text{CH}_2]$	$-\text{CH}_2$	$-\text{CH}_2$	$-\text{C}(\text{O})$	$-\text{O}-$	Energy (kcal/mol)
12.7	T	T	T	T	T	
11.9	T	T	T	\bar{E}	T	4.0
10.1	T	G	T	\bar{G}	T	-10.4
8.6-a	T	T	G	\bar{G}	T	-14.9
8.6-b	T	G	E	\bar{G}	T	-14.7
5.1	T	G	G	\bar{G}	T	3.7

^a A free P4HB chain of respectable energy (2.0 kcal mol⁻¹) with a torsional angle sequence of T_3ET_6 and a pitch of 12.2 Å was not included since it does not fulfil the symmetry conditions (2_1 helix) of the fibre diagram

elastomer of bacterial origin¹⁹. Its fibre diagram is illustrated in *Figure 5*. From the prominent meridional diffraction on the second layer line, it is possible to deduce a d spacing of 5.96 Å and, hence, a fibre repeat of 11.9 Å per two repeat units. The existence of even order reflections along the meridional suggests the presence of a 2_1 -axis along the crystallographic c -axis direction. An 'all trans' planar zigzag chain of P4HB was generated and subsequently minimized. The 'all trans' chain possesses a fibre repeat of 12.4 Å per two repeat units and it is evident that the discrepancy between this pitch value and the measured 11.9 Å is due to an inherent regular torsion along the chain backbone. The 'all trans' chain was consequently stretched to a high-energy state and allowed to relax along the fibre direction in order to produce energetically favourable free chain conformers with a pitch less than 12.4 Å. Following this procedure, five minimum-energy conformers were produced with the resulting conformations reported in *Table 4*.

Stable P4HB conformers were generated with fibre repeats of 5.1, 8.6, 10.1 and 11.9 Å, two with degenerate energies at 8.6 Å. The conformational arrangement of these five chains along with the 'all trans' chain is presented schematically in *Figure 6*. Of most importance is the conformer possessing a pitch of 11.9 Å given its excellent agreement with the observed fibre diagram pitch. It contains an eclipsed torsional angle directly next to the carbonyl of

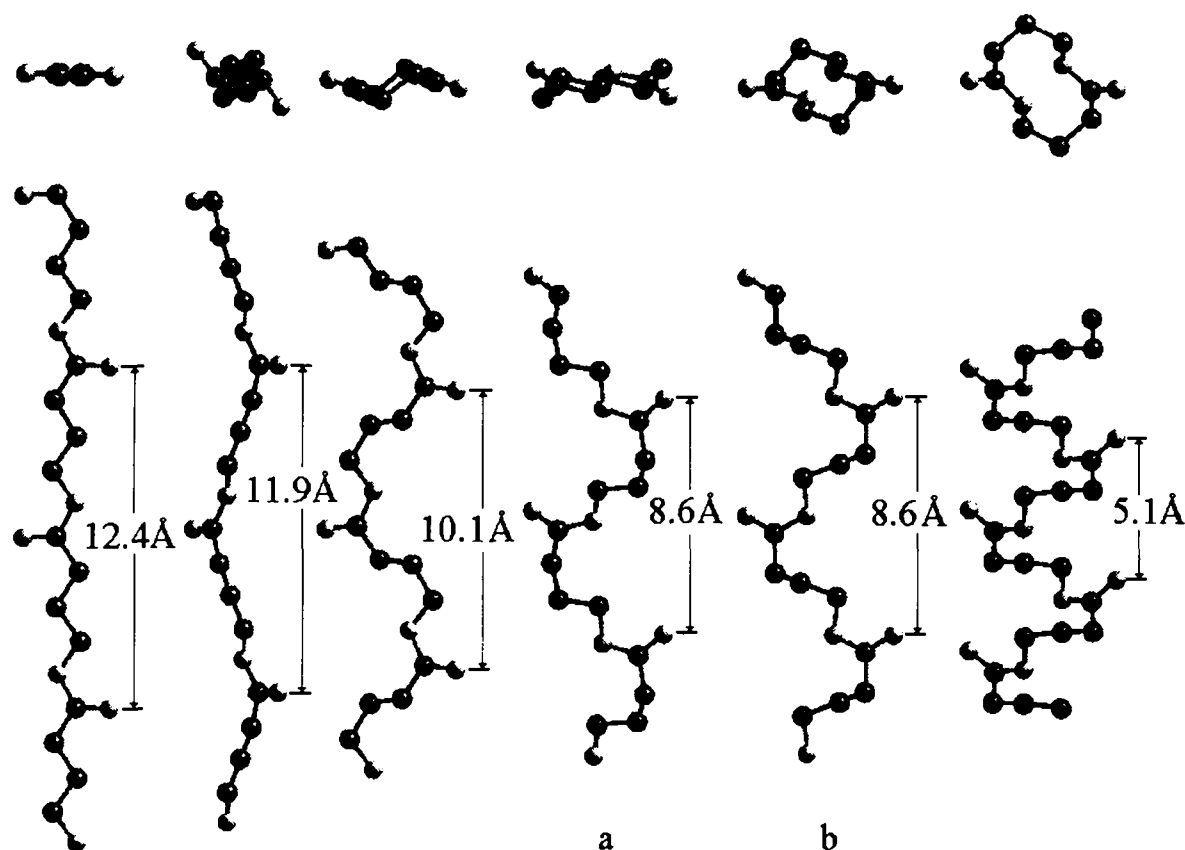


Figure 6 Orthogonal and down chain projections of a series of six modelled low-energy conformers of P4HB. The conformer possessing a pitch of 11.9 Å corresponds directly to the observed X-ray fibre repeat. All chains adopt a regular 2_1 helical conformation after energy minimization

the ester group, whereas the remaining torsional angles remain in the *trans* conformation. Upon decreasing the pitch, the helix cross section expands due to kinking which occurs in the two remaining bonds of the trimethylene sequence. This is energetically favourable up to a pitch value of 8.6 Å, then more energy is required to collapse the chain down to 5.1 Å. The two bonds within the ester moiety remain for all intents and purposes in the *trans* conformation even at such low pitches and the free chain keeps its regularly arranged 2_1 right-handed helix. This sort of conformational arrangement is akin to what is observed in PHB chains as they crystallize in regular repeating $(T_2G_2)_2$ helices with a 2_1 left-handed symmetry. Stretched P4HB is an example of a straight chain aliphatic polyester which does not exist in an 'all *trans*' planar zigzag conformation as reported for poly(β -propiolactone)²⁰. The insertion of regular torsion along the chain backbone is probably responsible for the short extension chain reversibility of this elastomer¹⁹. The next step in this study consists of packing the 11.9 Å conformer in a suitable crystal lattice respecting the *d* spacings, the symmetry elements and reflection intensities derivable from the well-oriented X-ray fibre diagram shown in Figure 5.

Poly(tetramethylene succinate)

Poly(tetramethylene succinate), otherwise known under the trade name of Bionolle[®], is a crystalline synthetic aliphatic polyester exhibiting excellent biodegradable properties⁸. It has been demonstrated recently that this polyester can be strained to induce an α - β reversible crystal modification²¹. Two crystal forms with definite fibre periods have been identified depending upon the magnitude of the applied strain. The α form in the original fibre has a period of 10.91 Å, whereas the strained β form has a fibre

period of 11.90 Å. The crystalline conformation of each of these forms was constructed by taking into account the observed fibre periods and by applying the bond lengths and angles appropriate for poly(ethylene succinate). A torsional angle sequence of T₇GTG² for the α form and an 'all *trans*' T₁₀ planar zigzag combination for the highly strained induced β modification was proposed. Cerius² modelling has been employed in this study to verify the possible crystalline conformations in this extended-chain biodegradable synthetic polyester.

A single, 'all *trans*' free chain of PTMS was constructed and minimized with respect to bonding terms, vdW and electrostatic interactions. The 'all *trans*' character of the torsional angles was retained during the calculation and the fibre repeat for the succinate and tetramethylene units together was found to be 12.4 Å, which is the same hypothetical distance evaluated for P4HB. Assuming the accuracy of this calculated value, it is likely that the strained β form of period 11.90 Å is not a fully extended planar zigzag chain and that some regular kinking is present. The 'all *trans*' chain (Cerius²) was subsequently used as a precursor in order to develop plausible conformers to explain the experimental α and β fibre periods. The PTMS chain was stretched to high energies and allowed to relax into minimum energy conformations by permitting displacement along the fibre direction. Other reasonable minimum energy conformers were found with fibre repeat distances of 12.2, 11.6 and 10.9 Å, and are illustrated in Figure 7. These distances correspond closely to the experimental ones for the α and β forms of PTMS. The torsional angles and energies of these resulting conformations along with the results of²¹ are summarized in Table 5.

Ichikawa *et al.* contend that kinking along the PTMS

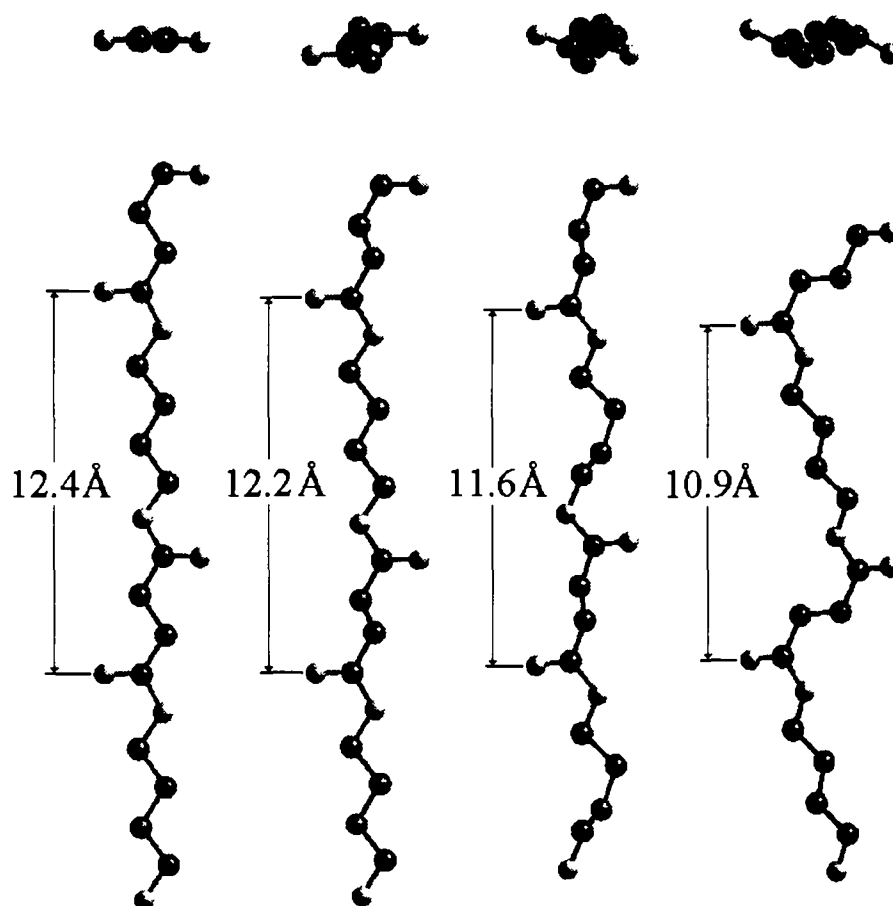


Figure 7 Orthogonal and down chain projections of the α - β crystalline conformers as well as the 'all *trans*' form of Bionolle[®]. Molecular modelling predicts three plausible crystalline conformers with pitches (10.9, 11.6 and 12.2 Å) close to the experimental ones (10.9 and 11.9 Å). The 'all *trans*' planar zigzag chain (12.4 Å) is energetically unfavourable but is similar in length to its equivalent in P4HB

Table 5 Pitches, torsional angles and energies (kcal mol^{-1}) of the single free PTMS chain for both the reported and modelled α and β crystalline forms

Pitch (Å)	[-CH ₂	-CH ₂	-CH ₂	-CH ₂	-O	-C(O)	-CH ₂	-CH ₂	C(O)	-O-]	Form	Energy (kcal mol^{-1})
12.4	T	T	T	T	T	T	T	T	T	T	all <i>trans</i>	-19.4
12.2	T	T	T	T	T	T	E	T	E	T	β	17.2
11.6	G	T	\bar{G}	T	T	T	E	T	\bar{E}	T	β	-12.5
10.9	T	T	T	T	T	T	G	T	\bar{G}	T	α	13.6
10.91 ^b	T	G	T	\bar{G}	T	T	T	T	T	T	α	-16.6 ^c

^aExperimental pitch of β form is 11.9 Å²¹

^bExperimentally observed pitch and proposed PTMS α conformation²¹

^cEnergy obtained for minimized Ichikawa *et al.* T-GTG free chain in Cerius² (pitch extends to 11.2 Å upon minimization)

backbone occurs preferentially in the tetramethylene unit in the α form. Using their proposed torsional angle sequence and performing an energy minimization, produced a conformer possessing a pitch of 11.2 Å and an energy of 16.6 kcal mol^{-1} . Our modelled α form of PTMS has a more reasonable pitch length (10.9 Å) and a comparable free chain energy (-13.6 kcal mol^{-1}). Kinking is preferred at both C(O)-CH₂ bonds within the succinate and not the tetramethylene unit. This conformation is supported by the earlier results on bacterial PHB and P4HB, where the barrier to torsional rotation about the C(O)-CH₂ bond is the lowest in aliphatic polyesters.

It has already been pointed out that the completely extended chain planar zigzag conformation is not plausible for the β form of PTMS. Two possible minimum energy conformers with a pitch of 11.6 and 12.2 Å, respectively, were obtained with repeat lengths relatively close to the

experimental fibre repeat distance of 11.9 Å. In both cases, the C(O)-CH₂ bonds of the succinate unit adopt an eclipsed conformation. In addition, two eclipsed conformations are observed within the tetramethylene unit of the 11.6 Å conformer. The 12.2 Å conformer is energetically more favourable compared with the 11.6 Å one, without taking into account energy packing considerations. All in all, free chain energies are quite similar between the modelled α and β forms of PTMS. The absence of an 'all *trans*' conformation in the β form of cold-stretched Bionolle[®] is in keeping with the earlier results observed for PHB and P4HB.

CONCLUSIONS

Molecular modelling energy packing considerations support the Brückner model as being the most reliable for depicting

the chain conformation and packing in iso-PHB. Molecular modelling suggests that S incorporation is isomorphous in all R iso-PHB free chain up to approximately 50% syndiotacticity. Molecular modelling energy calculations upon a free chain of PHB can be used to demonstrate why a theoretical 100% syndiotactic chain must have an expanded conformation with respect to the iso-PHB chain. It has been shown that the 'all trans' planar zigzag conformation of iso-PHB and syn-PHB is energetically unfavourable and that the extended state of side group containing aliphatic polyesters must include a regular sequence of kinking along the main chain backbone. From X-ray fibre diffraction data, an extended 2_1 helical chain conformation is proposed for P4HB possessing a pitch of 11.9 Å. Molecular modelling has useful prediction value as other feasible yet hypothetical low-energy conformers are suggested for P4HB with pitches approaching those of PHB. Three reliable energy-minimized conformers have been reported for stretched PTMS, satisfying the observed fibre repeat distances for the α (10.9 Å) and β (11.6 Å) crystalline forms. The short extension reversible elasticity which is present in strained P4HB and PTMS fibres is probably due to the regular kinking in the main chain backbone. Contrary to bacterial polyesters, certain biodegradable polyesters like PTMS are synthetic in origin, and degrade by the action of natural enzymes²².

This report stresses the importance of reliable crystal structural analyses with the further aim in mind to comprehend better the specific binding characteristics of depolymerase enzymes with respect to these extended conformations. Recent studies on depolymerase action on PHB single crystals have demonstrated that there is a correlation between the chain-folding crystal plane and the direction of chain scission^{23,24}. This is probably related to binding domains identified in PHB depolymerases²⁵ and the relatively fast hydrolysis of PHB crystalline areas. The same mechanism is likely for all crystalline poly(hydroxyalkanoates).

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REFERENCES

1. Lemoigne, M. C. R., *Acad. Sci.*, 1925, **180**, 1539; *Ann. Inst. Pasteur*, 1925, **39**, 144; *Bull. Soc. Chim. Biol.*, 1926, **8**, 770; *Ann. Inst. Pasteur*, 1927, **41**, 148.
2. Mark, H. and Whitby, G. S., ed., *The Collected Works of Wallace Hume Carothers*. Interscience, New York, 1940.
3. Holmes, P. H., in *Developments in Crystalline Polymers*, Vol. 2., ed. D. C. Bassett. Elsevier, New York, pp. 1–65.
4. Müller, H. M. and Seebach, D., *Angew. Chem. Int. Ed. Engl.*, 1993, **32**, 477.
5. Steinbüchel, A. and Valentin, H. E., *FEMS Microbiol. Lett.*, 1995, **128**, 219.
6. Yokouchi, M., Chatani, Y., Tadokoro, H., Teranishi, K. and Tani, H., *Polymer J.*, 1973, **14**, 267.
7. Hocking, P. J. and Marchessault, R. H., *Macromolecules*, 1995, **28**, 6401.
8. Yokoto, Y., Ishioka, R., Moteki, Y. and Watanabe, N., in *Biodegradable Plastics and Polymers*, ed. Y. Doi and K. Fukuda. Elsevier, Amsterdam, 1994, p. 577.
9. Abe, H., Matsubara, I., Doi, Y., Hori, Y. and Yamaguchi, A., *Macromolecules*, 1994, **27**, 6018.
10. Okamura, K. and Marchessault, R. H., in *Conformation of Biopolymers*. Vol. 2. ed. G. N. Ramachandran. Academic Press, London, 1967, p. 709.
11. Cornibert, J. and Marchessault, R. H., *J. Mol. Biol.*, 1972, **71**, 735.
12. Brückner, S., Meille, S. V., Malpezzi, L., Cesàro, A., Navarini, L. and Tombolini, R., *Macromolecules*, 1988, **21**, 967.
13. Hocking, P. J. and Marchessault, R. H., *Polym. Bull.*, 1993, **30**, 163.
14. Kemnitzer, J. E., McCarthy, S. P. and Gross, R. A., *Macromolecules*, 1992, **25**, 5927.
15. Hocking, P. J., Ph.D. thesis, Department of Chemistry, McGill University, 1995.
16. Ors, W. J., Marchessault, R. H., Bluhm, T. L. and Hamer, G. K., *Macromolecules*, 1990, **23**, 5368.
17. Prud'homme, R. E. and Marchessault, R. H., *Macromolecules*, 1974, **7**, 541.
18. U.S. Patent 1172619, 1969.
19. Doi, Y., *Microbial Polyesters*. VCH Publishers, New York, 1990.
20. Tadokoro, H., *Structure of Crystalline Polymers*. John Wiley, New York, 1979, p. 87.
21. Ichikawa, Y., Suzuki, J., Washiyama, J., Moteki, Y., Noguchi, K. and Okuyama, K., *Polymer*, 1994, **35**, 3338.
22. Nishioka, M., Tuzuki, T., Wanajyo, Y., Oonami, H. and Horiuchi, T., in *Biodegradable Plastics and Polymers*, ed. Y. Doi and K. Fukuda. Elsevier, Amsterdam, 1994, p. 584.
23. Hocking, P. J., Marchessault, R. H., Timmins, M. R., Lenz, R. W. and Fuller, R. C., *Macromolecules*, 1996, **29**, 2472.
24. Nobes, G. A. R., Marchessault, R. H., Chanzy, H., Briese, B. H. and Jendrossek, D., *Macromolecules*, 1996, **29**, 8330.
25. Jendrossek, D., Backhaus, M. and Andermann, M., *Can. J. Microbiol.*, 1995, **41**, 160.