Biosynthesis and Characterization of Deuterated Polyhydroxyoctanoate

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The synthesis of a polyhydroxyalkanoate with medium chain length alkyl substituents by *Pseudomonas oleovorans* was investigated using protonated and deuterated forms of octanoic acid in a minimal salts medium. Cultivation with deuterated octanoic acid resulted in a reduced rate of polymer accumulation compared to that with its protonated counterpart (107 and 207 mg of polymer L^{-1} of medium h^{-1} of cultivation, respectively). Nuclear magnetic resonance and gas chromatography coupled mass spectrometry of the derivatized polymer was used to establish the extent and distribution of deuterium in the biopolymer. A partially deuterated heteropolymer with 3-hydroxyoctanoic acid as the main constituent was produced. Deuteration is an important tool for contrast variation studies using neutron scattering, but predicates that the deuterated polymer is otherwise comparable in its physiochemical and material properties to its protonated counterpart. In studies reported here, the deuterated biopolymer exhibited an additional diffraction maximum at 7.55 Å and slight differences in its melting point (60 and 55 °C) and glass transition temperature (−39 and −36 °C) when compared to its protonated equivalent. While significant differences between the protonated and deuterated biopolymers were determined, our results support the use of this deuterated polyhydroxyalkanoate in its application in investigations using analytical neutron scattering techniques.

Introduction

Growth of a wide variety of microorganisms with an abundant carbon source and one or more limiting nutrients results in the intracellular accumulation of biopolyesters, "polyhydroxyalkanoates" (PHAs).[1] The metabolic flexibility of the PHA biosynthetic pathways has been harnessed to produce a variety of novel biopolymers possessing one or more of over a 100 different monomeric components.[2] These novel biopolymers possess material properties ranging from rigid and crystalline to amorphous and flexible.[1,3] Partly because of these properties, some of their members have been investigated as biodegradable alternatives to commercial petroleum-based thermoplastics and as medical implantation devices.[4]

The introduction of deuterium into such biomolecules is desirable inasmuch as it may allow more detailed analysis of polymer chain conformations and dynamics using small angle neutron scattering (SANS). However, the application of SANS to biological molecules is still an emerging technology with enormous potential.[5] In the presence of a neutron beam, the probability of a scattering event for a nucleus is provided by its isotopic scattering length, "*b*". The variation in scattering lengths between hydrogen (^{1}H) and deuterium (^{2}H) practically encompasses the range observed for all atoms (^{1}H $b = -3.742 \times 10^{-15}$ m, ^{2}H $b = 6.671 \times 10^{-15}$ m). It is this large negative *b* for hydrogen and its variation with its "heavy" counterpart that make neutron scattering an attractive technology for investigating biological systems. Thus, varying the deuterium content of the material and its surroundings permits the selective masking of structural components during neutron scattering. Such "contrast variation" experiments have been used to great effect to determine the conformation of polymers in microemulsions and their dynamics during the melt phase.[6,7] Deuteration of biological molecules, however, is somewhat limited to in vitro chemical deuteration through exchangeable sites and the in vivo deuteration of transgenic proteins produced in bacterial expression systems.[8] The synthesis of deuterated biological molecules presents a challenge that must be faced if analytical neutron techniques are to find wider application in biological systems.

The physiochemical and material properties of PHA-based devices can be manipulated through various techniques including copolymerizing and blending.[1,9] Contrast variation with SANS is an ideal technique to investigate the conformation and behavior of selective PHA components in such systems, but predicates that the deuterated biopolymer is otherwise comparable in its physiochemical properties to its hydrogenated counterpart. This has yet to be demonstrated.

Reports on the deuteration of PHAs are restricted to the works of Gross et al. and Yoshie et al. who successfully demonstrated the biodeuteration of poly(3-hydroxybutyrate) using *Rhodobacter sphaeroides* and *Alcaligenes eutrophus*.[10,11] Poly(3-hydroxybutyrate) (PHB) is the most studied member of the PHA family and is a homopolymer comprised of the monomer β-hydroxybutyric acid (HBA). HBA is also one of the ketone

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bodies produced in mammalian systems.[12] As a consequence of its biocompatibility, PHB has been studied as a biomaterial for medical implantation devices.[13,14] However, the relatively high crystallinity and brittle nature of PHB, when processed through various melt crystallization or solvent-casting techniques, severely limits its potential applications. In contrast, poly(3-hydroxyoctanoate) (PHO) is a heteropolymer composed of C6, C8, and C10 monomers and is significantly more amorphous and flexible than PHB. This biopolymer has also demonstrated biocompatibility with mammalian cells in in vitro tests.[15]

In this paper we report the synthesis of partially deuterated PHO through fed-batch cultivation of *Pseudomonas oleovorans* using perdeuterated octanoic acid as a carbon source. Analysis using NMR and GC-MS provided information on the distribution of deuterium in the biopolymer. Furthermore, the physiochemical and mechanical properties of solvent-cast films of PHO and its deuterated counterpart are compared.

Materials and Methods

Materials. Octanoic acid was purchased from Sigma-Aldrich (Sydney, Australia). Octanoic-d15 acid was purchased from Isotec (Sydney, Australia). All other chemicals were of at least 98% purity and obtained from APS Chemicals (Seven Hills, Australia).

Bacterial Strains, Inoculum Preparation, and Growth Conditions. Stock cultures of *P. oleovorans* (ATCC 29347) were stored at −20 °C from which working cultures were obtained.[16] The bacteria were cultivated aerobically in a 500 mL baffled Erlenmeyer flask containing 100 mL of modified E* medium and 20 mM octanoic acid (30 °C, 200 rpm). Modified E* medium consisted of the following: $(NH_4)HPO_4$, 5.94 g/L; K_2HPO_4, 5.8 g/L; KH_2PO_4, 3.7 g/L; 100 mM $MgSO_4$, 20 mL/L; and micro-element solution, 1 mL/L. The micro-element solution contained 2.78 g/L $FeSO_4 \cdot 7H_2O$, 1.98 g/L $MnCl_2 \cdot 4H_2O$, 2.81 g/L $CoSO_4 \cdot 7H_2O$, 1.67 g/L $CaCl_2 \cdot 2H_2O$, 0.117 g/L $CuCl_2 \cdot 2H_2O$, and 0.29 g/L $ZnSO_4$ dissolved in 1 M HCl. At late growth phase (as determined by OD_{660}) the flask contents were used to inoculate four flasks with the same conditions. These were then cumulatively used as an inoculum for a 5 L fermentor containing 3.6 L of modified E* medium and 20 mM octanoic acid (final operating volume 4 L, Biostat-B, Braun Scientific, Germany). Biomass was grown at 30 °C with constant forced aeration until the octanoic acid was exhausted; the dissolved oxygen tension (DOT) was subsequently maintained at <5% through the addition of 533 mM octanoic acid. Additional aliquots of the substrate were provided when a rise in DOT indicated substrate depletion. The same protocol was simultaneously carried out but with the fed-batch stages of the cultivation using perdeuterated substrate (octanoic-d15 acid).

Polymer Extraction. Ten milliliter samples were periodically taken during cultivation and centrifuged (6000*g*, 15 min) prior to washing in sterile fresh medium with no carbon sources. The biomass was then lyophilized and the dry weight calculated. At completion of cultivation, the intracellular polymer was extracted from the lyophilized cell mass through chloroform and purified by repeat methanol precipitation.[16]

Gas Chromatography-Mass Spectrometry. PHO concentration was determined using GC-MS after methanolysis of the lyophilized biomass (HP5890 series II GC coupled with a VG TRIO-1 MS, Hewlett-Packard, PA, USA). The GC was equipped with a BPX5 capillary column (25 m/0.25 mm i.d./0.25 μm film, SGE, Sydney, Australia) and helium was used as carrier gas (1.2 mL/min). The operating protocol was as follows: 30 °C for 3 min; then the temperature was raised to 250 °C (20 °C/min) and maintained there for 5 min. One microliter injections were made with a split ratio of 10:1. PHO composition was identified and quantified based on retention times against known standards and *m*/*e* values.[16] Methyl esters of the PHO were subsequently derivatized to pyrrolidides and GC-MS used to investigate the extent and location of deuterated atoms in the monomer units.[17]

Nuclear Magnetic Resonance Spectroscopy (NMR). Samples of the biopolymers were dissolved in deuterated chloroform (ca. 4 mg/mL) and examined using a Bruker DMX600, operating at 600.13 MHz for ^{1}H NMR (5 mm tube, 5 mm probe). Samples were also dissolved in chloroform and examined at 46.07 MHz using a Bruker DPX-300 for ^{2}H NMR (5 mm tube, 10 mm probe). Spectra were recorded with a pulse width of 4.5 μs (45° pulse), a spectral width of 6.6 kHz, an acquisition time of 2.5 s, a relaxation delay of 6 s with 64−256 scans for required signal-to-noise, and referenced internally to $CHCl_3$ and $CDCl_3$ (7.26 ppm with respect to tetramethylsilane).

Gel Permeation Chromatography GPC analyses of polymers were conducted using an LC-10ATVP Shimadzu solvent delivery system coupled to a SIL-10ADVP Shimadzu auto-injector (±1 μL). A column set consisting of a PL 5.0 μm bead size guard and 3−5.0 μm PL linear columns (10e3, 10e4, and 10e5 Å) were maintained at 40 °C (CTO-10AC VP Shimadzu Column Oven). A RID-10A Shimadzu Refractive Index Detector was used. Tetrahydrofuran (THF) was utilized as the continuous phase (flow rate: 1 mL/min). A series of polystyrene standards possessing low polydispersity (<1.1) were used to generate a calibration curve.

Thermal Analysis. The polymers were investigated using a Perkin-Elmer differential scanning calorimeter (DSC-7). Samples weighing approximately 7 mg in closed pans were heated at the rate of 20 °C/min from −80 to 127 °C.[18] The data obtained was used to calculate the glass transition temperatures (T_g), melting point (T_m), and fusion enthalpies (ΔH_f). Means of three samples were determined. Thermogravimetric analysis of the polymers was performed using a Perkin-Elmer Pyris 1 TGA under a nitrogen atmosphere with a heating rate of 10 °C/min to 320 °C followed by an increase to 600 °C at a heating rate of 40 °C/min.

X-ray Diffractometry and Material Properties. X-ray diffraction analysis of solvent-cast films was performed using a Siemens Diffraktometer D5000 generator operating at 30 kV and 30 mA. Monocrom Kα radiation (λ = 1.5418 Å) was used. The morphologies of PHO and dPHO solvent-cast films were visualized using scanning electron microscopy.

The material properties of solvent-cast films of the biopolymers were examined using an Instron Mini (Instron MA). Twenty-four samples of PHO and 7 dPHO were clamped to the calibrated tensiometer with pneumatic grips which moved apart at 22 mm/min until failure, maximum load, and extension to break were monitored and the tensile strain, stress, and Young's modulus statistically determined.[19]

Results and Discussion

***P. oleovorans* Growth Studies.** Deuteration of PHAs is limited to the studies by Gross et al. and Yoshie et al. reporting the synthesis of partially deuterated PHB in cells of *R. sphaeroides* and *A. eutrophus*.[10,11] However, flexibility in PHA synthesis is species-dependent. Thus, whereas these species synthesize PHB, a PHA with relatively short length constituents in the side chain (*scl*PHA), *P. oleovorans* can produce PHAs with medium length alkyl side chains (*mcl*PHAs) such as PHO, as well as PHAs incorporating chemically functional groups (*fcl*PHAs), for example, polyhydroxyphenylvalerate (PHPV).

Figure 1 illustrates the changes in biomass, cell, and polymer concentrations, during fed-batch cultivation of *P. oleovorans* on protonated and deuterated octanoic acid with water as the liquid phase. Polymer accumulation by *P. oleovorans* grown on perdeuterated octanoic acid was approximately half that of the culture that was supplied with feeds of its protonated counterpart (107 and 207 mg of polymer L^{-1} of medium h^{-1} of cultivation, respectively). These differences may have been due to acclimatization of the species to the deuterated substrate. This is consistent with the report of Foster, demonstrating the deuterated substrates can affect metabolic pathways in biological

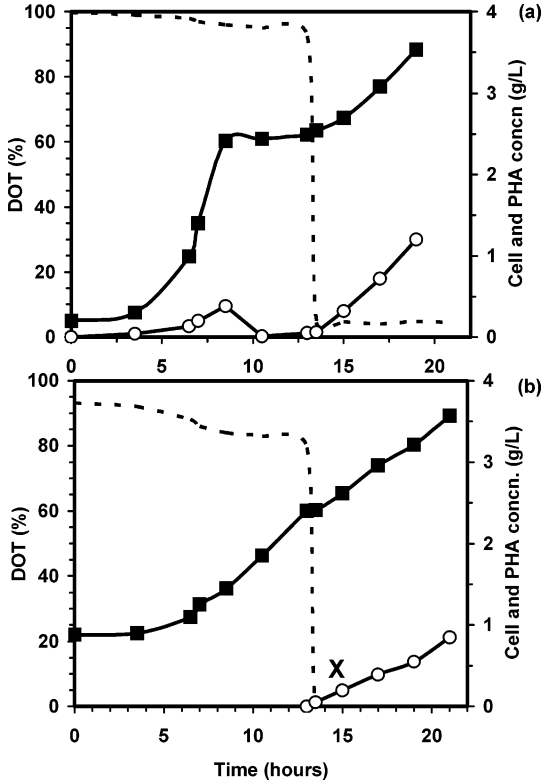

Figure 1. Biomass accumulation (■) and production of PHA (○) in *P. oleovorans* cultivated in E* medium with (a) protonated and (b) protonated followed by feeds of perdeuterated octanoic acid, (---) dissolved oxygen tension, and (X) initiation of feeds of carbon substrate linked to dissolved oxygen tension.

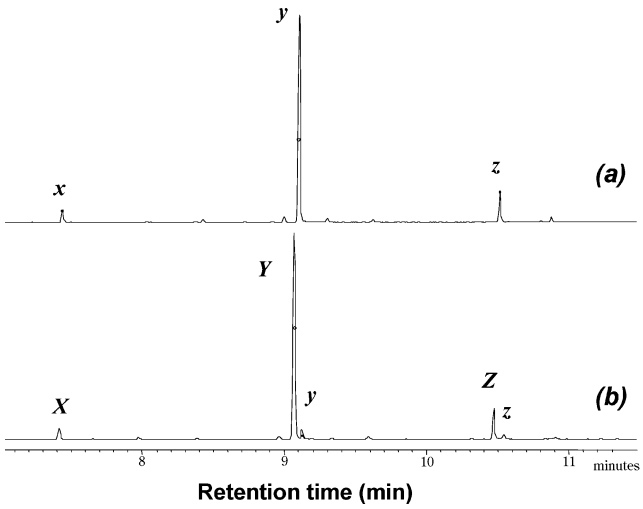

Figure 2. GC spectra of methanolyzed PHA extracted from *P. oleovorans* cultivated on protonated (a) and (b) deuterated substrates, illustrating different retention times for protonated x−z and deuterated monomeric components X−Z.

systems, but contrasts with previous studies by Katz and Crespi and Gross et al.[10,20,21]

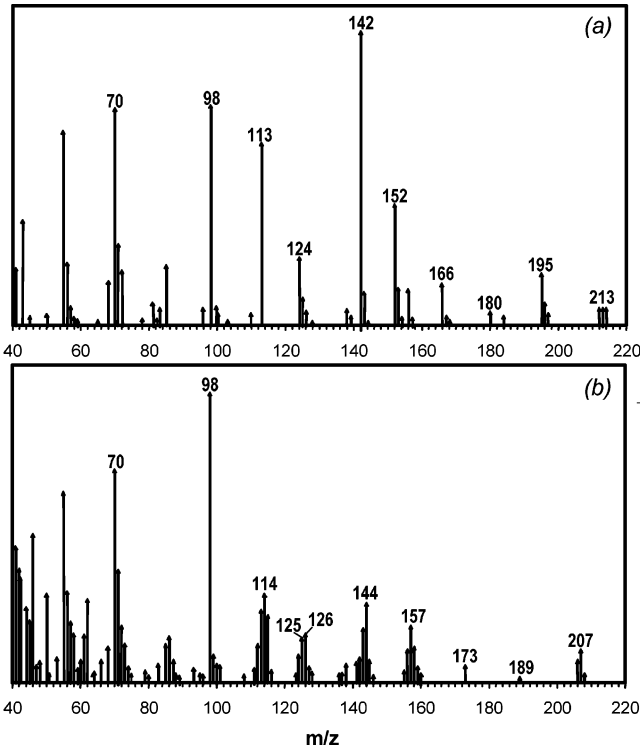

Figure 3. GC spectra of pyrrolidide derivatives of PHA extracted from *P. oleovorans* cultivated on protonated (a) and (b) deuterated carbon substrates, illustrating different masses due to deuteration.

GC-MS Analysis. The GC spectra for methanolyzed monomers of the polymer extracted from *P. oleovorans* initially cultivated on octanoic acid and then batch-fed with perdeuterated octanoic acid differed from that cultivated on octanoic acid alone (Figure 2). When cultivated on octanoic acid, *P. oleovorans* synthesized the *mcl*PHA, poly(3-hydroxyoctanoate) (PHO). PHO from this fermentation actually possessed three monomeric components, with 79% being hydroxyoctanoate (HO-y), 14% hydroxyhexanoate (HH-z), and 7% hydroxydecanoate (HD-x). Polymer extracted from *P. oleovorans* fed with octanoic-d15 acid also displayed three peaks corresponding to the monomeric methyl esters but these were observed with slightly displaced retention times (HD-X, HO-Y, HH-Z), suggesting greater masses. In addition, small peaks matching the retention times for HO (y) and HH (z) were also present, indicating that the polymer possessed both hydrogenated and deuterated monomeric components (Figure 2 and Table 1). This may be due to residual octanoic acid from the batch phase, or metabolism of accumulated PHO.

The methyl esters of the monomeric components were further derivatized to their pyrrolidides to confirm the location and extent of deuteration. The GC-MS spectrum for derivatized PHO shows a noticeable peak representing a mass of 70, corresponding to the amide ring of the pyrrolidide ion. The presence of a ketone group (C1) increases this mass to 98 and a peak for this is also readily observed (Figures 3a and 4a). The peak representing a mass of 113 corresponds to the presence of a

Table 1. Composition, Molar Masses, and Thermal and Material Properties for PHO and dPHO

	composition (mol %)						Mn	Mw		stress		Young's
PHA	C6	C8	C10	T_g (°C)	T_m (°C)	ΔH_m (J/g)	($\times 10^3$)[a]	($\times 10^3$)[a]	PDI	(MPa)[b]	strain	modulus (MPa)
PHO	14 ± 2	79 ± 2	7 ± 2	−35.8 ± 0.6	54.7 ± 1.2	23.8 ± 3.1	142	235	1.65	6.5	5.8	5.7
dPHO	13 ± 3	83 ± 4	4 ± 2	−39.0 ± 0.8	60.1 ± 1.1	23.1 ± 2.9	107	197	1.84	10.5	6.6	7.1

[a] Molar masses were determined by GPC. [b] SD for stress, strain, and Young's modulus for PHO = 1960, 1.0, and 1.9; dPHO = 2787, 1.9, and 1.3.

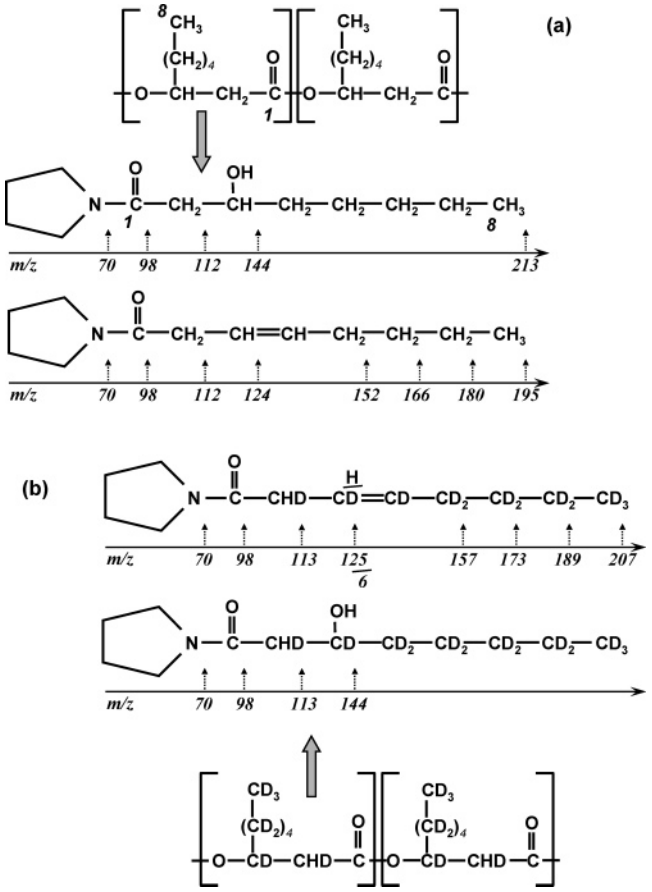

Figure 4. Structural interpretation GC-MS data for pyrrolidide derivatives of *mcl*PHAs purified from P. oleovorans cultivated on protonated (a) and (b) deuterated carbon substrates.

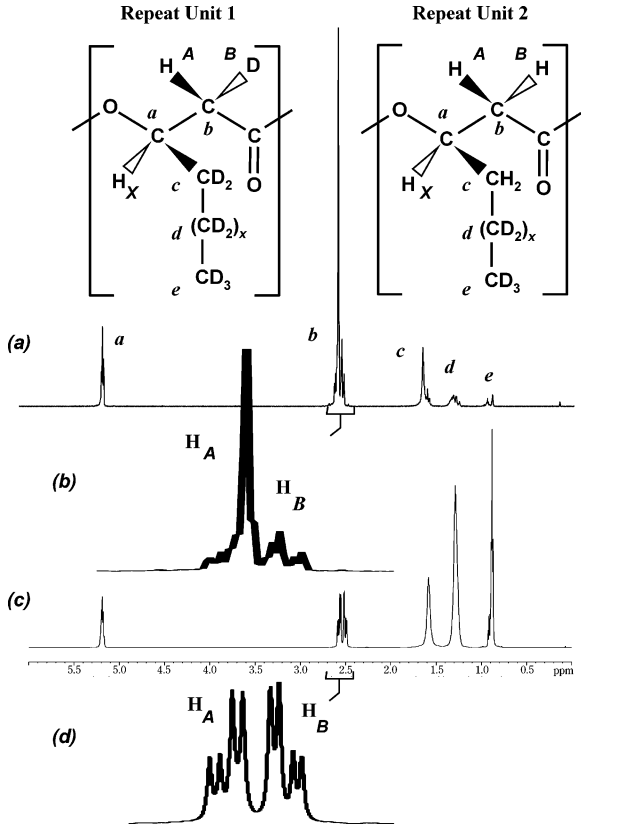

Figure 5. 600 MHZ ^{1}H NMR spectra of *mcl*PHA purified from *P. oleovorans* cultivated with (a, b) deuterated and (c, d) protonated carbon substrates, (b, d) expansion of spectral regions at 2.4−2.7 ppm, with proposed chemical structures of monomeric repeat units for dPHO.

CH_2 group, from C2 of the monomer; which actually provides a mass of 112 but interaction with an adjacent proton gives the slightly higher value (Figures 4a and 5a). In contrast, the PHO extracted from *P. oleovorans* fed with octanoic-d15 acid showed a peak representing a mass of 114, indicating that a proton of this C2 atom was replaced by a deuteron, confirming the synthesis of partially deuterated PHO, "dPHO" (Figures 3b and 4b).

The third carbon of the HO monomer forms a CHOH group in its derivative; this has a molecular mass of 30 which is displayed as a peak corresponding to a mass of 142 in the derivative (Figure 4a). However, this C3 atom tends to form a double bond with the C2 with the loss of two hydrogens and an oxygen (18 mass units), also giving a signal at 124 mass units (Figure 4a). In contrast, the pyrrolidide derivative of dPHO displays a mass of 144; this represents an increase of 31 mass units to the partially deuterated backbone (113). In this case, the addition of the OH group is consistent with the derivatization process; thus, the proton of the C3 atom was also replaced by a deuteron (Figures 3b and 4b). The formation of a double bond between the C2 and C3 atoms reduces this mass to either 125 or 126 depending upon whether the loss from C2 is a hydrogen or deuterium atom (Figures 3b and 4b).

The derivatized hydroxyoctanoate ion should exhibit a total mass of 213, but this is observed as only a weak signal. The peak representing mass 195 corresponds to this HO ion with a loss of water (18 mass units). Similarly, peaks within this derivative representing the mass units at C7 (180), C6 (166), and C5 (152) are clearly present (Figures 3a and 4a). Derivatives for dPHO display similar peaks, with the total HO ion corresponding to a mass of 207, C7 (189), C6 (173), and C5 (157), indicating deuteration of the side chain in the PHO. Thus, GC-MS analyses of the pyrrolidide derivatives of PHO extracted from *P. oleovorans* fed-batch with deuterated substrate suggested complete deuteration of the monomeric components, with the exception of single protons at the C2 positions.

NMR Analysis. The ^{1}H NMR spectra of the dPHO extracted from *P. oleovorans* showed patterns of ^{1}H−^{1}H coupling similar to those generated by its protonated counterpart, indicating a level of nondeuterated repeat units (Figure 5). This is consistent with the GC analysis of the methanolyzed derivatives (Figure 2). Assuming Bernoullian characteristics, it is clear that the ratios of peak areas for PHO are not consistent with those of its partially deuterated counterpart dPHO. In particular, the intensity of peaks "*d*" and "*e*" attributed to protons in the aliphatic side chain were greatly reduced, suggesting the presence of monomeric components possessing various degrees of deuteration.

An expansion of the spectrum showing the methylene region at the C2 atom for PHO is shown in Figures 5c and 5d. The ^{1}H−^{1}H coupling between H_A, H_B, and H_X results in eight peaks and is analyzed as an ABX type (Figure 5d).[21] The relatively low intensity of the methine hydrogen H_X in the NMR spectrum for the partially deuterated PHO is consistent with the GC-MS studies, suggesting a high level of deuteration in this position (Figure 5a). This suggestion is further supported by the comparative reduction in vicinal coupling between H_X and the diastereotopic methylene hydrogens, H_A and H_B in the spectrum obtained for dPHO (Figure 5b). The obvious disparity in peak intensity for these methylene hydrogens corroborates the GC-MS analysis which suggested that deuterium replaced only one of the methylene hydrogens.

The combination of NMR analysis of the biopolymers and GC-MS analysis of their derivatized monomeric components suggests that the partially deuterated PHO, (dPHO), consists of three components. The predominant component of this biopolymer consists of monomeric units where all protons, with the exception of a single methylene hydrogen at C2, have been replaced by deuterons. The second component is comprised of monomers exhibiting varying degrees of deuteration, with the side chain effectively deuterated, while the final minor component is protonated PHO.

The monomeric components of dPHO can be explained as a result of the fed-batch process where the cultivation of *P. oleovorans* on an initial concentration of octanoic acid led to the synthesis of PHO. As the substrate was gradually depleted, it reached a threshold value where this intracellular polymer was significantly degraded.[22] Under the cultivation conditions used here, this degradation was observed in real time as an increase in dissolved oxygen tension, with the consequential addition of deuterated substrate. Therefore, at this point there was a mixture of protonated and deuterated substrates, together with partially degraded PHO. Furthermore, Doi and co-workers have shown that both intracellular PHA synthesis and degradation occur during polymer accumulation.[1] Biosynthesis of deuterated PHB also produced polymer with monomers displaying various degrees of deuteration.[10,11]

The predominant component of the dPHO consisted of fully deuterated monomers with the exception of one of the methylene hydrogens at the C2 atom. This is consistent with the metabolic pathway for *mcl*PHAs synthesis in Pseudomonads belonging to the RNA homology group I.[23] By the same biochemistry, the minor component of PHO originates from protonated substrate. The biochemical origin of the monomers exhibiting varying degrees of deuteration requires further clarification and is currently under investigation. It is clear, however, that in addition to facilitating SANS studies, biodeuteration can also be utilized independently as an investigative tool for elucidating biochemical pathways.[11]

Physiochemical and Material Properties. Alterations to polymer production regime can result in changes in PHA composition and molecular mass.[25] In these studies, the partially deuterated PHO showed little difference in monomer composition, but displayed noticeable changes in its molecular properties. dPHO had a lower weight average molecular weight (197 000) and greater polydispersity (1.84) compared to its PHO counterpart (235 000 Mw, 142 g/mol, and 1.65 PDI, Table 1). These differences in molecular mass are a consequence of differences in the fermentation systems and may include the influence of substrate deuteration.

The melting point of dPHO, as determined by DSC, was 60 °C, approximately 5 °C greater than its protonated counterpart. In addition, its glass transition temperature was also lower, −39 °C for dPHO compared with −36 °C for PHO (Table 1, Figure 6). Thermogravimetric analysis also showed a noticeable decrease in the deuterated biopolymer's thermal decomposition temperature, 251 ± 4 °C compared with 265 ± 4 °C for PHO (Figure 7). In contrast, there was no significant difference in the thermal enthalpies of the two biopolymers, 24 and 23 J g^{-1} for PHO and dPHO, respectively (Table 1).

The thermodynamic properties of a polymer are affected by a number of factors including molecular weight and composition. A significant reduction in molecular mass (~16%) for dPHO compared to that for PHO introduced a greater degree of free volume into the polymer chains. However, this change in free volume was also mediated by the polymer density, which

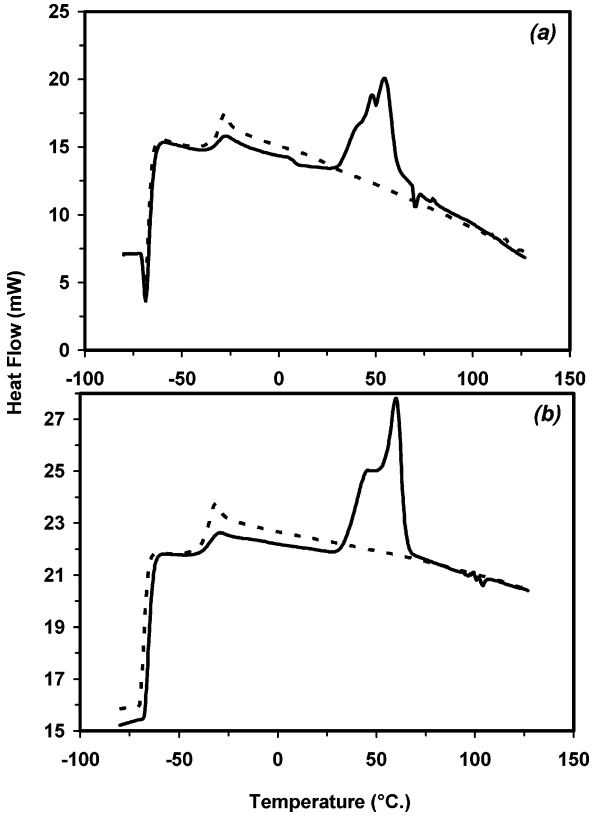

Figure 6. DSC thermographs of PHO (a) and dPHO (b), (−) heating trace, (---) cooling trace.

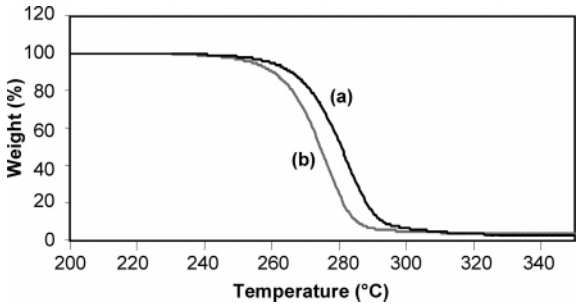

Figure 7. TGA thermograms for PHO (a) and dPHO (b).

increased due to deuteration. Similarly, small differences in the distribution of substituents in the side chain of dPHO may also have affected mobility of the polymer chains, influencing its melting point and glass transition temperature.

The differences in thermal properties between protonated and perdeuterated PHO may have also been influenced by the substitution of hydrogens by deuterons. While it has generally been assumed that deuteration of polymer chains has no effect on their thermodynamics, recent work by Kayillo et al. and others have shown that these properties are sensitive to deuteration.[26,27] The vibrational states of protonated and deuterated molecules are significantly different as they depend on the mass of their nuclei.[28] Due to its larger mass, the vibrational amplitude for deuterium is smaller than that for its protonated analogues, resulting in lower molar volumes and bond polarizability. Thus, the significantly greater polarizability of the C−H bond compared to that of the C−D bond results in increased intermolecular forces.[29]

The differences in molecular properties of dPHO and PHO were also manifested in the crystalline structure of solvent-cast films when analyzed using X-ray diffractometry (Figure 8). The interplanar *d* spacings were similar for both biopolymers with

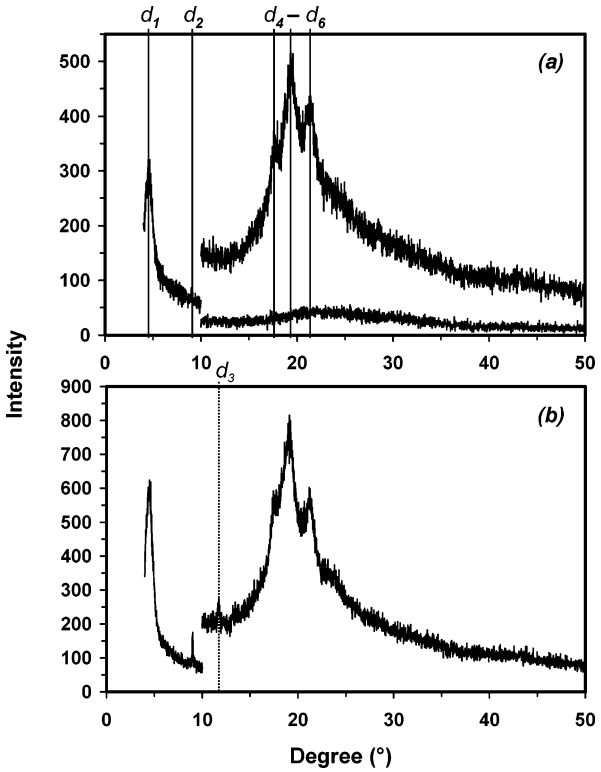

Figure 8. X-ray diffraction spectra for solvent-cast films of PHO (a) and dPHO (b).

Table 2. X-ray Diffraction Maxima for PHO and dPHO produced by P. *oleovorans*

	diffraction maxima (Å)					
sample	d_1	d_2	d_3	d_4	d_5	d_6
PHO	19.13	9.82		5.01	4.57	4.17
dPHO	19.63	9.80	7.55	5.04	4.66	4.19

some noticeable exceptions; the diffraction maxima for dPHO differed slightly from those exhibited by PHO and the deuterated biopolymer also displayed an additional diffraction spacing at 7.55 Å (Table 2, Figure 8). The diffraction pattern for PHO reported in this study differs from that reported by Gross et al. for PHO produced by *P. oleovorans* cultivated on the sodium salt of octanoic acid.[25] Similarly, Fuller and co-workers have reported significant changes in polymer composition based on changes to cultivation conditions, despite consisting of the same species and media.[30] Thus, differences in the diffraction patterns between PHO and dPHO are consistent with the biodeuteration process. Despite differences in their diffraction patterns, both dPHO and PHO possessed a similar crystallinity of approximately 31 ± 4%.

Solvent-cast films of dPHO and PHO showed no noticeable morphological differences when examined using SEM; however, the deuteration process appeared to strengthen the dPHO film. While increases in the tensile stress/strain at break and the Young's modulus were observed for films of dPHO compared to those of PHO, these changes were not statistically significant in this study (Table 1).

Conclusions

Processes for biodeuteration are still in their infancy. Deuteration of PHO through the cultivation of *P. oleovorans* on a deuterated carbon substrate and characterization of the resulting dPHO extract reveal a polymer with a high level of incorporation of deuterium, as demonstrated by a combination of analytical methods. Moreover, the deuterated biopolymer is otherwise comparable in its physiochemical properties to its protonated counterpart with slight but significant differences. Nevertheless, this outcome will allow the dynamics and conformation of the deuterated biopolymer to be investigated using SANS which will form the basis of further reports.

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