CHAPTER 2

Physical characteristics of poly(3-hydroxyalkanoates) and poly(3-hydroxyalkenoates) produced by *Pseudomonas oleovorans* grown on aliphatic hydrocarbons

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Physical Characteristics of Poly(3-hydroxyalkanoates) and Poly(3-hydroxyalkenoates) Produced by *Pseudomonas oleovorans* Grown on Aliphatic Hydrocarbons

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ABSTRACT: *Pseudomonas oleovorans* is able to accumulate poly(3-hydroxyalkanoates) (PHAs) after growth on *n*-alkanes and 1-alkenes. The composition and physical characteristics of these polyesters were shown to be substrate dependent. When *n*-alkanes (*n*-hexane to *n*-decane) were used, PHAs were formed consisting of 3-hydroxyalkanoate monomers of which the pendant group varied from a methyl to a heptyl group (saturated PHAs). When 1-alkenes (1-octene and 1-decene) were used as the carbon source, the polyester consisted of both 3-hydroxyalkanoate and terminally unsaturated 3-hydroxyalkenoate monomers, of which the pendant group varied in length between a propyl and a heptyl group (unsaturated PHAs). The structure of the PHAs was confirmed by ^1H and ^{13}C NMR. Apart from the PHAs isolated from *n*-hexane- and 1-alkene-grown cells, the copolymers were partly crystalline (ΔH_m = 6.6–18.7 J/g), having T_m's which varied between 38.9 and 58.5 °C and T_g's ranging from −30.8 to −39.7 °C. The T_g's of the amorphous polymers ranged from −25.8 to −43.1 °C. The M_w's of the isolated polymers ranged from 178 000 to 330 000.

Introduction

Poly(3-hydroxyalkanoates) (PHAs) are optically active polyesters that serve as storage material for bacteria.[1] Poly(3-hydroxybutyrate) (PHB), the best known example of these biopolymers, has been extensively studied since its discovery by Lemoigne in 1926.[2] PHB is synthesized by a wide range of bacteria and may be accumulated to up to 80% of the bacterial cell dry weight. It is a highly crystalline and brittle material. Since for many applications nonbrittle materials are likely to have more potential, efforts have been made to develop or to find PHB-related and other bacterial copolymers. Thus, 3-hydroxyvalerate or 4-hydroxybutyrate monomers have been incorporated into PHB variants by altering the growth substrate of PHB-synthesizing microorganisms.[3,4] Moreover, PHA copolymers have been sought and identified in natural environments such as marine sediments and activated sludge.[5,6]

We have found that PHAs other than PHB are accumulated by *Pseudomonas oleovorans* during growth on *n*-alkanes in two-phase fermentations.[7] The precise composition of these polyesters is dependent on the alkanes in the apolar phase.[8] Alkane oxidation products such as alkanols, alkanals, and alkanoic acids can also be utilized by *P. oleovorans*[9-11] and other fluorescent pseudomonads[11,12] to synthesize a range of PHAs. In general, the *Pseudomonas* polymers are characterized by an alkyl group that varies from a propyl to a nonyl group depending on the substrate used (Figure 1).

1-Alkenes are also oxidized by *P. oleovorans* and yield poly(3-hydroxyalkenoates), which contain both unsaturated and saturated monomers.[8] The degree of unsaturation could be varied from 0 to about 50% by using varying ratios of *n*-alkanes and 1-alkenes as substrate. Gross et al.[10] have studied the physical characteristics of some of the PHAs that are formed when *P. oleovorans* is grown on fatty acids. We have studied the polymers formed on the corresponding *n*-alkanes, as well as those formed on several 1-alkenes.

In this paper we report on the composition and physical characteristics of these polymers and compare those to the composition and characteristics reported by Gross et al.[10] for PHAs formed from *n*-alkanoate sodium salts.

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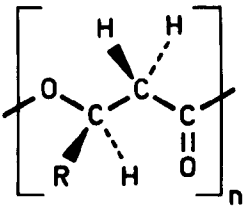

Figure 1. General structural formula of PHAs produced by *P. oleovorans* after growth on linear alkanes. The R group varies from a propyl to a nonyl group depending on the carbon source used to grow the organism.[8]

Experimental Section

PHA Accumulation and Isolation. The polyesters were isolated from *P. oleovorans* (ATCC 29347). Cells were precultured overnight at 30 °C in 250-mL Erlenmeyer flasks containing 50 mL of E-medium[13] supplemented with 0.1% (v/v) MT microelements[8] and 2% (v/v) *n*-alkane. Three of the resulting precultures were used to inoculate a 10-L BIOSTAT E fermentor (B. Braun, FRG), which contained 9 L of E2-medium[8] supplemented with 1 mM $MgSO_4 \cdot 7H_2O$, 0.1% (v/v) MT microelements, and 10% (v/v) *n*-alkane (*n*-hexane to *n*-decane) or 1-alkene (1-octene or 1-decene), which was the only carbon and energy source present. In all cases, the stirrer speed was kept constant at 600 rpm and the dissolved oxygen tension was maintained above 60% air saturation. The starting cell density was about 0.01 g/L dry weight. The cells were harvested (Sharpless continuous centrifuge) about 10 h after the culture had entered the stationary phase and freeze dried.

The lyophilized cells were ground and suspended in chloroform (5 g cells in 500 mL of chloroform). The suspension was refluxed during 4 h, after which it was cooled down to room temperature and filtered. The filtrate was reduced to about 100 mL by evaporation and filtered again, after which it was slowly added to 1 L of 96% ethanol under stirring. Stirring was stopped, and after the gellike material had settled, the ethanol/chloroform mixture was decanted. The precipitate was dissolved again in chloroform (20 mL). This solution was filtered and precipitated again in a 10-fold volume of 96% ethanol. The resulting precipitate was dissolved in chloroform, after which the volume was reduced to 5–10 mL by evaporation. After complete evaporation of chloroform, a polymer film was obtained, which was stored in a vacuum exsiccator until further analysis.

Analytical Procedures. GLC analysis was performed on a capillary gas chromatograph (Packard, Downers Grove, IL)

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Table I
Relative Monomer Composition of the PHAs Produced by *Pseudomonas oleovorans* Grown on *n*-Alkanes as the Sole Carbon and Energy Source

substrate	rel monomer compos of PHA,[a] mol %						
	HB	HV	HC	HH	HO	HN	HD
n-hexane			83.1 ± 0.5	<1.0	12.0 ± 0.2	<1.0	4.9 ± 0.4
n-heptane		2.5 ± 0.1		97.5 ± 0.1	<1.0	<1.0	
n-octane	<1.0		12.2 ± 0.2		87.8 ± 0.2		<1.0
n-nonane		2.3 ± 0.1		40.6 ± 0.4	1.4 ± 0.1	55.7 ± 0.5	
n-decane	<1.0		11.1 ± 0.4	1.1 ± 0.1	65.8 ± 0.3	1.2 ± 0.1	20.8 ± 0.7

[a] HB = 3-hydroxybutyrate, HV = 3-hydroxyvalerate, HC = 3-hydroxycaproate (=3-hydroxyhexanoate), HH = 3-hydroxyheptanoate, HO = 3-hydroxyoctanoate, HN = 3-hydroxynonanoate, and HD = 3-hydroxydecanoate.

equipped with a 25-m CP-Sil5CB capillary column (Chrompack, The Netherlands). Samples were injected by split injection. For peak analysis, capillary gas chromatography–mass spectrometry (GC–MS) was performed on a Ribermag R 10-10 C apparatus equipped with an identical column. The samples were ionized by electron impact (70 eV) and by chemical ionization using NH_3 as the reactant gas.

^1H NMR spectra were recorded on a Varian VXR 300 NMR spectrometer operating at 300 MHz. Samples were prepared in chloroform-*d* (20–30 mg/mL). The spectra were recorded at ambient temperature (20 °C) with 24.0-μs pulse width and 2.0-s relaxation delay. The 75-MHz ^{13}C NMR spectra were recorded at 20 °C on a 20–30 mg/mL $CDCl_3$ solution. We used an APT (attached proton test) pulse sequence with a τ delay of 0.007 s, which gives the quaternaries and CH_2's up and the CH_3's and CH's down. The 90° pulse width was 8.0 μs, and the relaxation time was 3.0 s.

Melting points and glass transition temperatures were recorded on a Perkin-Elmer DSC7. Samples of 10–15 mg were heated at 10 °C/min from −100 to +100 °C.

Molecular weight data were obtained by gel permeation chromatography (Waters 150-C ALC/GPC). Polystyrene standards with a low polydispersity (purchased from Polymer Laboratories, U.K.) were used to obtain a calibration curve.

Results and Discussion

Production of Poly(3-hydroxyalkanoates). Poly(3-hydroxyalkanoates) (PHAs) were isolated from *P. oleovorans* after growth on various *n*-alkanes ranging from *n*-hexane to *n*-decane. The PHA yields for the *n*-octane, *n*-nonane, and *n*-decane cultivations were in the range of 18–26% of the cell dry weight, and when *n*-hexane or *n*-heptane was used as growth substrate, the amount of polymer isolated from the cells was in the range of 1–5%. The composition of the polyesters was determined by GLC analysis of the methanolyzed polymers as described by Lageveen et al.[8]

When *n*-alkanes were used as the sole carbon source, copolymers were formed, the composition of which is given in Table I. Each copolymer preparation was methanolyzed in duplicate, and each of the resulting methyl ester solutions was analyzed by GLC in triplicate. The average compositions thus determined showed standard deviations that never exceeded 9% and were generally below 4%. The *n*-octane-derived copolymer was prepared four times, and all preparations had the composition shown in Table I. The identity of 3-hydroxy fatty acids was confirmed by GC–MS analysis of the methanolyzed polymeric material by using both electron impact and chemical ionization mass spectrometry.

The polymers that were accumulated after growth on *n*-alkanes showed a preference for the incorporation of 3-hydroxyoctanoate or 3-hydroxynonanoate monomers depending on whether a C-even- or a C-odd-numbered *n*-alkane was used. The relative monomer composition of the PHAs isolated from the *n*-heptane-, *n*-octane-, *n*-nonane, and *n*-decane-grown cells was not significantly different from the data we have reported in an earlier study.[14]

Table II
Comparison of the Relative Monomer Composition of the PHAs Produced by *Pseudomonas oleovorans* after Growth on *n*-Octane and Sodium Octanoate

substrate	rel monomer compos of PHA,[a] mol %							ref
	HB	HV	HC	HH	HO	HN	HD	
n-octane			12		88		<1	this study
			13		87			Lageveen[8]
octanoate			10		89		1	Huisman[11]
			10		86		4	Brandl[9]
	2		7		85		6	Gross[10]

[a] See Table I.

However, when *n*-hexane was used as the carbon and energy source, a copolymer was accumulated instead of a homopolymer as we reported previously.[8] This copolymer contained 12.0 mol % 3-hydroxyoctanoate, 4.9 mol % 3-hydroxydecanoate, and traces of 3-hydroxyheptanoate and 3-hydroxynonanoate in addition to 83.1 mol % 3-hydroxyhexanoate.

The composition of the different PHAs was independent of the growth phase of the cells. For example, when *P. oleovorans* was grown in a biphasic system with *n*-octane as the carbon source, the PHA isolated at 11 different times always consisted of 87.8 mol % 3-hydroxyoctanoate, 12.2 mol % 3-hydroxyhexanoate, and traces of 3-hydroxydecanoate.

PHAs can be synthesized by *P. oleovorans* when the cells are grown on linear alkanes or their respective oxidation products. Thus, PHAs formed after growth of *P. oleovorans* on *n*-alkanes, alkanols, and alkanoic acids have been studied.[8-11] It might be expected that the composition of PHAs formed by *P. oleovorans* grown on a given substrate should be independent of its oxidation state. This was in fact observed in our laboratory for PHAs formed by *P. oleovorans* after growth on either *n*-alkanes (ref 8 and this paper) or the corresponding *n*-alkanoate sodium salts.[11] As an example, Table II compares the composition of the PHAs formed after growth of *P. oleovorans* on *n*-octane and octanoic acid.

Gross et al. and Brandl et al. have also studied PHAs formed after growth of *P. oleovorans* on *n*-alkanoate sodium salts. The monomer composition of these PHAs differs somewhat from those studied in our laboratory, as illustrated in Table II for PHAs formed after growth of *P. oleovorans* on sodium octanoate, in that they also contain 3-hydroxydecanoic acid monomers.

Production of Poly(3-hydroxyalkenoates). Poly(3-hydroxyalkenoates) were isolated from 1-octene- and 1-decene-grown cells. The yields of the unsaturated polymers were 10 and 2% for the 1-octene- and 1-decene-grown cells, respectively. These polymers consist of both unsaturated and saturated monomers (Table III). In general we can conclude that, for these C-even substrates, saturated and unsaturated C-even monomers were incorporated into PHAs.

Table III
Relative Monomer Composition of the Poly(3-hydroxyalkenoates) Produced by *Pseudomonas oleovorans* When 1-Alkenes Were Used as Substrate

substrate	rel monomer composn of PHA,[a] mol %							
	HC	HCU	HO	HOU	HN	HNU	HD	HDU
1-octene	7.2 ± 0.4	5.4 ± 0.3	41.5 ± 0.6	45.9 ± 0.4			<1.0	<1.0
1-decene	8.1 ± 0.4	3.0 ± 0.2	28.8 ± 0.2	33.5 ± 0.6	1.6 ± 0.1	<1.0	6.6 ± 0.4	18.5 ± 0.6

[a] HCU = 3-hydroxyhexenoate, HOU = 3-hydroxyoctenoate, HNU = 3-hydroxynonenoate, and HDU = 3-hydroxydecenoate.

The presence of unsaturated monomers was expected because alkane hydroxylase can oxidize the saturated end of alkenes to yield ω-hydroxy-1-alkenes, which can be metabolized further to the corresponding fatty acids. These are degraded by β-oxidation, yielding unsaturated 3-hydroxyacyl–CoA intermediates, which are used in the polymerization reaction.[8] However, the presence of saturated monomers in PHAs isolated from cells grown on 1-alkenes was not expected. Alkene terminal double bonds are oxidized by *P. oleovorans* to the corresponding 1,2-epoxyalkanes, which accumulate extracellularly or react with cellular proteins.[15] The fact that PHAs formed by cells grown on alkenes also contain saturated monomers could be due to contamination of the alkenes used as substrates with alkanes. This trivial explanation is unlikely, however, based on analysis of the alkenes prior to use and on experiments where known amounts of alkanes were added to alkenes to determine how substrate mixtures affect the composition of PHAs formed by *P. oleovorans*.[8] These experiments showed that contamination of alkenes with alkanes could not account for the number of saturated monomers found in PHAs formed by growth of *P. oleovorans* on alkenes. It appears therefore that alkene double bonds are oxidized to carboxyl groups, either by oxidation of the epoxides formed by alkane hydroxylase or by direct oxidation of alkenes to alkanals.[16] The alkanal will then be oxidized further to a saturated fatty acid, which is converted to the saturated acyl–CoA intermediate while the unsaturated alkanol will yield the unsaturated acyl–CoA intermediate. It can be tentatively concluded from the composition of the unsaturated PHAs (Table III) that both reactions are about equally likely. As was the case for PHAs formed from saturated alkanes, the PHAs formed from 1-octene and 1-decene contained only C-even monomers, again pointing to the removal of acetate units only from the acyl–CoA intermediates.

^{1}H NMR Analysis. The poly(3-hydroxyalkanoates) and poly(3-hydroxyalkenoates) were analyzed by ^{1}H NMR. Figure 2 shows characteristic 300-MHz ^{1}H NMR spectra of the PHAs isolated from *P. oleovorans* grown either on saturated (*n*-octane) or unsaturated (1-octene) substrates. The saturated polymers (*n*-alkanes as the carbon source) all show spectra with the same chemical shifts also seen in the spectrum of the *n*-octane-derived PHA (Figure 2A). Since the composition of the saturated polymers depends on the monomer chain length, peak d (Figure 2A), which reflects the mean side-chain length of the monomeric units, varies with the carbon source used to produce PHA. Integration of peak d yields mean side-chain lengths that are close to those calculated from the GC data presented in this work (Table IV). The ^{1}H NMR spectra of the saturated PHAs closely resemble the spectra of the PHAs isolated on the corresponding *n*-alkanoate sodium salts, as described by Gross et al.[10]

The 300-MHz ^{1}H NMR spectrum of the PHA produced after growth of *P. oleovorans* on 1-octene is shown in Figure 2B together with the chemical shift assignments for each proton. This spectrum shows additional resonances

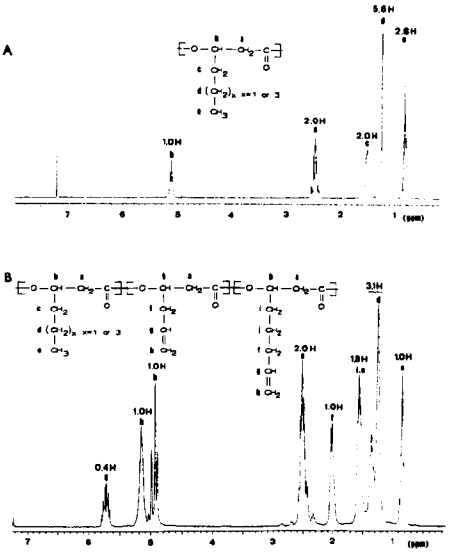

Figure 2. 300-MHz ^{1}H NMR spectra of PHAs isolated from *P. oleovorans*: (A) PHA isolated from *n*-octane-grown cells; (B) PHA isolated from 1-octene-grown cells.

Table IV
Average Number of Methylene Protons[a] in the Different Pendant Groups of PHA Monomers

substrate	GC data	^{1}H NMR data
n-hexane	2.9 H	3.1 H
n-heptane	3.9 H	3.8 H
n-octane	5.5 H	5.6 H
n-nonane	6.2 H	6.0 H
n-decane	6.4 H	6.2 H

[a] Protons in the pendant group of the PHA monomers that were assigned to peak d (Figure 2).

compared to the "*n*-octane" spectrum at 5.73, 4.92, 2.02, and 1.36 ppm due to the presence of the terminal unsaturated group in 51% of the monomers. The chemical shifts of the other protons are similar to the ones observed in Figure 2A. The ratio of the integration of peak f to peak a of 0.52 indicates the fraction of unsaturated monomers in the polymer and agrees very well with the value of 0.51 calculated from the GC data. The "1-decene" 300-MHz ^{1}H NMR spectrum resembles the spectrum of the unsaturated polymer shown in Figure 2B: resonances appear at identical chemical shifts, but as expected the integration of the peaks that were assigned to the protons in the unsaturated pendant group are different. As was the case for the PHA formed by 1-octene-grown cells, the area ratio of peaks f to a (0.57) agreed very well with the value of 0.55 calculated from the GC data.

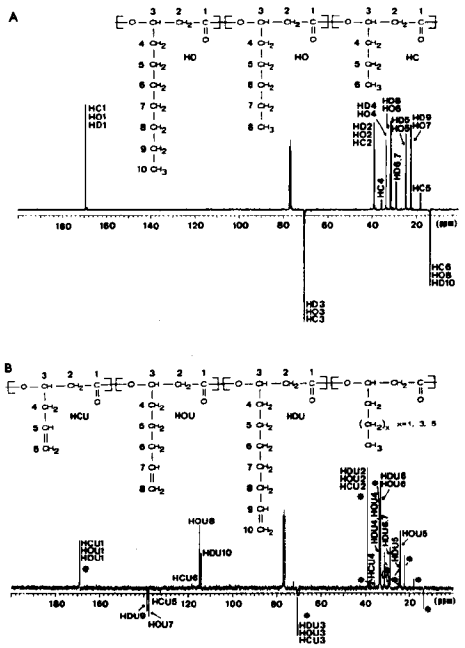

Figure 3. 75-MHz ^{13}C NMR spectra of PHAs isolated from *P. oleovorans*. Only carbon atoms of the major monomer units (relative amount > 1.2%) were assigned: (A) PHA isolated from *n*-decane-grown cells; (B) PHA isolated from 1-decene-grown cells. Peaks marked with an asterisk were (partly) due to the presence of saturated 3-hydroxyalkanoate monomers (HD, HO, and HC) in this polymer. These resonances were already assigned in (A).

^{13}C NMR Analysis. The whole set of described polymers was subjected to 75-MHz ^{13}C NMR analysis. Figure 3 shows the spectra of the PHAs isolated from *n*-decane- and 1-decene-grown cells.

Saturated PHAs. Peaks were assigned by comparing these spectra for all of our PHA samples. Major peaks were assigned by associating them with major monomer types and by using tables of chemical shifts for *n*-alkanes.[17] The resulting assignments were compared with the chemical shifts for the carbon atoms of the monomeric units of the PHAs isolated from *n*-alkanoate-grown cells as reported previously.[10] The saturated PHAs all show a similar spectrum apart from the number of peaks between 18 and 36 ppm and the peaks corresponding to the methine and methyl carbon. The carbonyl carbon resonances of the different saturated PHA monomers were not resolved in distinct peaks and appeared at a chemical shift of 169.22 ± 0.05 ppm. The main-chain methylene carbon resonances were also not resolved. The chemical shift of this carbon was found at 39.01 ± 0.06 ppm for the different PHA monomers. The chemical shift of the methine carbon was 70.72 ± 0.07 ppm for monomers with a pendant group longer than a propyl, while it was found 0.24 ppm upfield from the unresolved peak for the 3-hydroxyhexanoate monomer. The methyl carbon resonances of the different PHA monomers were all resolved in distinct peaks. The chemical shift of the methyl carbon of the longest pendant group was 13.97 ± 0.01 ppm, while the methyl carbon of the shorter pendant groups showed resonances upfield.

The major difference between the spectra of the saturated PHAs occurs in the region between 18 and 36 ppm. This cluster of resonances is due to the different methylene carbon atoms in the different PHA pendant groups. These different methylene carbons are all resolved in distinct peaks and show chemical shifts comparable to those described by Gross et al.[10]

Unsaturated PHAs. These PHAs were produced by using either 1-octene or 1-decene as the carbon source. The ^{13}C NMR spectra of these PHAs showed additional resonances at 114.22, 114.89, and 118.68 ppm due to the terminal methylene carbon in the pendant group and at 132.47, 137.93, and 138.67 ppm due to the methine carbon of the terminal double bond, compared to the spectra discussed above for the saturated PHAs. Some additional resonances were also found in the region 18–36 ppm. These extra resonances were assigned to the methylene carbon atoms in the pendant group of the unsaturated monomers after comparison of the ^{13}C NMR spectra to the spectra of the other PHA samples and after the influence of the double bond on the chemical shift of the adjacent methylene carbons was established by using known chemical shifts of linear *n*-alkanes and 1-alkenes.[17]

Properties of Poly(3-hydroxyalkanoates). Table V shows several physical properties of both the saturated and unsaturated PHAs. The melting points (T_m) of the saturated PHAs vary between 38.9 and 58.5 °C and are much lower than that of PHB (179 °C). The "hexane" polymer, being completely amorphous, does not show a melting endotherm. The corresponding enthalpies of fusion (ΔH_m) are also much lower relative to that of PHB, indicating that we are dealing with low-crystallinity polymers. Moreover, the glass transition temperatures (T_g) range from −25.8 to −39.7 °C and are considerably lower than the T_g of quenched PHB, which was determined to be 2.1 °C (result not shown). As a result of the low crystallinity and the low T_g, PHAs are flexible materials (except for the "hexane" polymer), while PHB is a strong, rigid material.

The weight-average molecular weights (M_w) of the saturated PHAs vary between 178 000 and 330 000 and are comparable to that of PHB.[1] It was found that the polymer that was produced in the highest amount, the "octane" polymer, had the lowest M_w and M_n, while the polymers that are produced in lower amounts ("hexane" and "heptane" polymers) had the highest M_w and M_n. This corresponds to a degree of polymerization for the PHAs isolated from *n*-hexane- and *n*-heptane-grown cells that is about twice as high as the DP values of the other three polymers (Table V). The same phenomenom was observed by Gross et al.[10] Whether there is a relation between the degree of polymerization and the intracellular amount of PHA remains to be determined.

Although the monomeric composition of the PHAs isolated from *P. oleovorans* grown on *n*-alkanes differs slightly from that of PHAs isolated by Gross from *n*-alkanoate-grown cells,[10] the T_m, T_g, ΔH_m, and molecular weights of corresponding PHAs are essentially identical. Thus, the differences in composition between the polymers isolated by Gross et al. and the corresponding PHAs studied here are too small to be expressed in their physical properties. Referring to the ΔH_m values of the various PHAs, we can conclude that the presence of pentyl, hexyl, and/or heptyl pendant groups in the monomers favor polymer crystallinity.

Table V also shows the characteristics of the two poly(3-hydroxyalkenoates). These copolymers are both

Table V
Physical Characteristics of the Saturated and Unsaturated PHAs Isolated from *P. oleovorans* after Growth on n-Alkanes or 1-Alkenes, Respectively

substrate	T_g, °C	T_m, °C	ΔH_m, J/g	M_w	M_n	D (M_w/M_n)	DP
n-hexane	-25.8			330 000	182 000	1.8	1490
n-heptane	-30.8	38.9	6.6	308 000	160 000	1.9	1258
n-octane	-36.5	58.5	18.7	178 000	99 000	1.8	709
n-nonane	-39.7	47.8	14.2	240 000	131 000	1.8	879
n-decane	-38.4	47.6	15.4	225 000	113 000	2.0	766
1-octene	-36.6			242 000	101 000	2.4	730
1-decene	-43.1			260 000	117 000	2.2	804

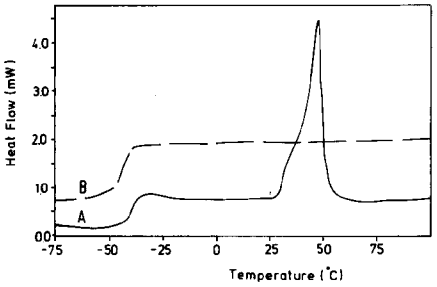

Figure 4. DSC thermograms of PHAs isolated from *n*-decane- (A) and 1-decene-grown (B) cells.

amorphous, having T_g's that are comparable to the T_g's of the corresponding poly(3-hydroxyalkanoates), which were isolated from *P. oleovorans* grown on either *n*-octane or *n*-decane. Although the poly(3-hydroxyalkenoates) contain mainly monomers with pentyl and/or heptyl pendant groups, similar to the corresponding "octane" and "decane" polymers, they do not show a melting endotherm. This is demonstrated in Figure 4, which shows the DSC thermograms of the PHAs isolated from *n*-decane- and 1-decene-grown *P. oleovorans* cells. The saturated PHA (Figure 4A) clearly shows a melting endotherm at 47.6 °C, which is not observed in the thermogram of the unsaturated PHA (Figure 4B). Both polyesters do, however, show a comparable glass transition temperature. Apparently the presence of unsaturated rather than saturated end groups somehow prevents a crystalline arrangement of the polymeric chains resulting in an amorphous polymer.

The M_w's of the unsaturated PHAs are higher than the M_w's of the saturated variants. The M_n's, however, are similar for both types of polymers. Since the mean length of the pendant group in the corresponding saturated and unsaturated PHAs are similar, the DP values for these PHAs are also similar.

Conclusions

In this paper we have studied PHAs formed by *P. oleovorans* grown on *n*-alkanes and 1-alkenes and related their physical characteristics to their composition. It was shown that the PHAs consisted of mainly C-even 3-hydroxyalkanoic acids when they were isolated from cells grown on C-even substrates and of mainly C-odd 3-hydroxyalkanoic acids when they were derived from C-odd carbon sources. The saturated PHAs were crystalline polymers except for the PHA isolated from *n*-hexane-grown cells. This amorphous polyester and the very low crystallinity PHA isolated from *n*-heptane-grown cells were accumulated in the lowest amounts and had the highest degree of polymerization. The PHAs with a higher degree of crystallinity, which were produced in a higher amount had a DP of half the value found for the low-crystallinity PHAs.

PHAs formed during growth of *P. oleovorans* on 1-alkenes consisted of monomers having a terminally unsaturated bond in 50% of the monomers. These polyesters consisted of at least four major different monomers. In contrast to PHAs isolated from the corresponding saturated carbon sources, which consisted of at most three different major monomers, they did not show a melting endotherm when subjected to differential scanning calorimetry. The presence of terminal double bonds in biological polyesters permits a variety of chemical and UV-induced modifications, which result in altered polymer characteristics (van der Galiën, J. G.; Preusting, H.; Witholt, B., experiments in progress).

More work will be necessary for the formulation of a satisfactory model of PHA synthesis and structure, which accounts for the physical properties of isolated PHAs. For now, it can be concluded that the presence of more than three major monomer types results in PHAs that are either amorphous or show very low crystallinity.

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