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^{13}C Nuclear Magnetic Resonance Studies of *Pseudomonas putida* Fatty Acid Metabolic Routes Involved in Poly(3-Hydroxyalkanoate) Synthesis

GERN N. M. HUIJBERTS, THEO C. DE RIJK, PIETER DE WAARD, AND GERRIT EGGINK*

Agrotechnological Research Institute (ATO-DLO), 6700 AA Wageningen, The Netherlands

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The formation of poly(3-hydroxyalkanoates) (PHAs) in *Pseudomonas putida* KT2442 from various carbon sources was studied by ^{13}C nuclear magnetic resonance spectroscopy, gas chromatography, and gas chromatography-mass spectroscopy. By using [1-^{13}C]decanoate, the relation between beta-oxidation and PHA formation was confirmed. The labeling pattern in PHAs synthesized from [1-^{13}C]acetate corresponded to the formation of PHAs via de novo fatty acid biosynthesis. Studies with specific inhibitors of the fatty acid metabolic pathways demonstrated that beta-oxidation and de novo fatty acid biosynthesis function independently in PHA formation. Analysis of PHAs derived from [1-^{13}C]hexanoate showed that both fatty acid metabolic routes can function simultaneously in the synthesis of PHA. Furthermore, evidence is presented that during growth on medium-chain-length fatty acids, PHA precursors can be generated by elongation of these fatty acids with an acetyl coenzyme A molecule, presumably by a reverse action of 3-ketothiolase.

Fluorescent *Pseudomonas* strains belonging to RNA homology group I are able to accumulate poly(3-hydroxyalkanoates) (PHAs), which consist of 3-hydroxy fatty acids, as a carbon and energy reserve (11, 18). The monomer compositions of these polyesters are variable and are determined by the specificity of the PHA polymerase system, the nature of the substrate, and the metabolic routes leading to PHA formation (5). On the basis of a structural analysis of PHAs synthesized by *Pseudomonas oleovorans* growing on medium-chain-length saturated and unsaturated alkanes, it has been proposed that 3-hydroxyacyl coenzyme A (acyl-CoA) intermediates of the beta-oxidation route are channeled to PHA synthesis (13). In line with these results, de Waard et al. (3) showed that the structure of the monomer units in PHAs formed by *Pseudomonas putida* KT2442 during growth on (poly)unsaturated long-chain fatty acids matches the structures that can be predicted from the beta-oxidation pathway for unsaturated fatty acids (16).

Detailed one- and two-dimensional nuclear magnetic resonance (NMR) studies on PHA synthesized from carbohydrates revealed the presence of both saturated and unsaturated 3-hydroxy fatty acids. The chemical structures of these constituents are identical to the structures of the acyl moieties of the 3-hydroxy-acyl acyl carrier protein (ACP) intermediates of the fatty acid biosynthetic pathway (10). In addition, it was demonstrated that the degree of unsaturation of PHA and membrane lipids is similarly influenced by shifts in cultivation temperature. On the basis of these results, we proposed that during growth on carbohydrates, intermediates of the de novo fatty acid biosynthetic pathway are diverted to PHA synthesis. It remained, however, to be determined whether PHA precursors are (in part) generated by beta-oxidation of newly synthesized long-chain fatty acids.

In order to establish in more detail the relation between PHA synthesis and fatty acid metabolism in *P. putida*, we studied PHA formation by using ^{13}C-labeled substrates and specific inhibitors of the fatty acid metabolic pathways. This approach allowed us to determine that in *P. putida*, both beta-oxidation and de novo fatty acid biosynthesis can function independently and simultaneously in generating precursors for PHA synthesis.

* Corresponding author. Mailing address: Agrotechnological Research Institute (ATO-DLO), P.O. Box 17, 6700 AA Wageningen, The Netherlands. Phone: 31-8370-75112. Fax: 31-8370-12260.

MATERIALS AND METHODS

Media and culture conditions. *P. putida* KT2442 (1) was used in all experiments. *P. putida* KT2442 was cultivated in 1-liter Erlenmeyer shake flasks containing 250 ml of 0.5× E2 medium (10) at 30°C in an orbital incubator at 150 rpm (Gallenkamp) with various carbon sources.

^{13}C-labeled fatty acids were added to the cultures at the end of the exponential growth phase. The substrate concentrations used were as follows: 10 mM decanoate plus 5 mM [1-^{13}C]decanoate and 20 mM hexanoate plus 5 mM [1-^{13}C]hexanoate. [1-^{13}C]acetate (0.6 g/liter) was added to cultures growing on 15-g/liter glucose at the end of the exponential growth phase. Cells were harvested by centrifugation (10 min at 5,000 × *g*), washed, and lyophilized. ^{13}C-labeled substrates were obtained from Isotec, Miamisburg, Ohio. Hexanoate and decanoate were obtained from Merck, Darmstadt, Federal Republic of Germany.

Polymer isolation and analysis. Isolation of PHA was performed by chloroform extraction of lyophilized cells followed by methanol precipitation of PHA. Gas chromatography (GC) analyses of PHA were performed as described previously (10). GC-mass spectroscopy (GC-MS) analyses were performed with an HRGC/MS GC attached by direct interface to a QMD 1000 ms mass spectrometer (both from Carlo-Erba, Milan, Italy). Spectra were obtained as electron impact (EI) spectra (70 eV) or as chemical ionization spectra, with isobutane as the reacting agent. The scan rate was 1 s^{-1}.

^{13}C NMR analysis was performed on PHA samples dissolved in deuterated chloroform. ^{13}C NMR spectra were recorded on a Bruker AC200 NMR spectrometer at a probe temperature of 27°C.

Inhibition of fatty acid metabolism. Inhibitors of beta-oxidation and fatty acid synthesis were acrylic acid (17) (Merck) and cerulenin (6, 12) (Sigma, St. Louis, Mo.), respec-

tively. In E2 medium with octanoate (10 mM) as the sole source of carbon and energy, the growth of *P. putida* KT2442 was inhibited by acrylic acid at a concentration of 0.1 mg/ml. Cerulenin inhibited the growth of *P. putida* KT2442 on mineral E2 medium at a concentration of 0.3 mg/ml.

RESULTS

PHA synthesis from [1-^{13}C]acetate. The relation between PHA formation and de novo fatty acid biosynthesis was studied by ^{13}C NMR and GC-MS analyses of PHAs synthesized from ^{13}C-labeled substrates. For this purpose, [1-^{13}C]glucose and [6-^{13}C]glucose are not suitable because the ^{13}C-labeled carbon atom is released as CO_2 in the reaction steps leading to acetyl-CoA. Therefore, we used [1-^{13}C]acetate, which after conversion to acetyl-CoA is a precursor for de novo fatty acid biosynthesis. Because *P. putida* KT2442 grows poorly on acetate as the sole carbon and energy source, we used glucose as the substrate with ^{13}C-labeled acetate as a cosubstrate. This results in a dilution of ^{13}C label so only a fraction of the carbon atoms in PHA are labeled. When *P. putida* KT2442 was cultivated on 1.5% glucose and 0.15% ^{13}C-labeled acetate, PHA formation was observed. GC analysis of the 3-hydroxy fatty acid methyl esters derived from purified polyester revealed that the monomeric composition did not differ from that of PHA isolated from *P. putida* KT2442 cultivated with glucose as the sole carbon source.

EI GC-MS showed that all 3-hydroxy fatty acid methyl esters derived from PHA synthesized from glucose–[1-^{13}C]acetate were enriched with ^{13}C. The ^{13}C NMR spectrum of PHA purified from *P. putida* KT2442 cultivated on glucose–[1-^{13}C]acetate is shown in Fig. 1, and the carbon chemical shift data are summarized in Table 1. The assignment of the signals in this spectrum is in agreement with earlier NMR studies (3, 4, 7, 9, 15). Compared with the signals of the even-numbered carbon atoms, all signals corresponding to odd-numbered carbon atoms are enhanced, indicating a ^{13}C enrichment of the odd-numbered carbon atoms.

Synthesis of ^{13}C-labeled PHA from [1-^{13}C]decanoate. To investigate the relation between fatty acid degradation and PHA synthesis, PHA formed from 1-^{13}C-fatty acids was analyzed by GC-MS and ^{13}C NMR. The compositions of PHAs isolated from *P. putida* KT2442 grown on [1-^{13}C]decanoate and on unlabeled decanoate are shown in Table 2. The ^{13}C NMR spectrum of PHA purified from *P. putida* KT2442 cultivated on [1-^{13}C]decanoate is depicted in Fig. 1. From the intensities of the signals corresponding to carbon atoms 1 and 2 in PHA derived from [1-^{13}C]decanoate and unlabeled decanoate, we estimate a 10-fold ^{13}C enrichment of the C-1 carbon atom in PHA from [1-^{13}C]decanoate.

EI GC-MS analysis of the 3-hydroxy fatty acid methyl esters derived from PHA formed on [1-^{13}C]decanoate showed that only the 3-hydroxydecanoate methyl esters were enriched. On the basis of the EI GC-MS spectra (Fig. 2), it was also determined that enrichment was exclusively located in a fragment with an *m/e* of 103 corresponding to carbon atoms C-1, C-2, and C-3 of the monomers (7).

Synthesis of ^{13}C-labeled PHA from [1-^{13}C]hexanoate. The composition of PHA derived from [1-^{13}C]hexanoate and unlabeled hexanoate as determined by GC is shown in Table 2. In addition to the major constituent, 3-hydroxyhexanoate, six other monomer units were identified: 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, 3-hydroxydodecenoate, 3-hydroxytetradecanoate, and 3-hydroxytetradecenoate. These monomers are normally found in PHA isolated from *P. putida* KT2442 cells cultivated on non-PHA-related substrates (10). The presence of these monomers was further confirmed by ^{13}C NMR spectroscopy (Fig. 3).

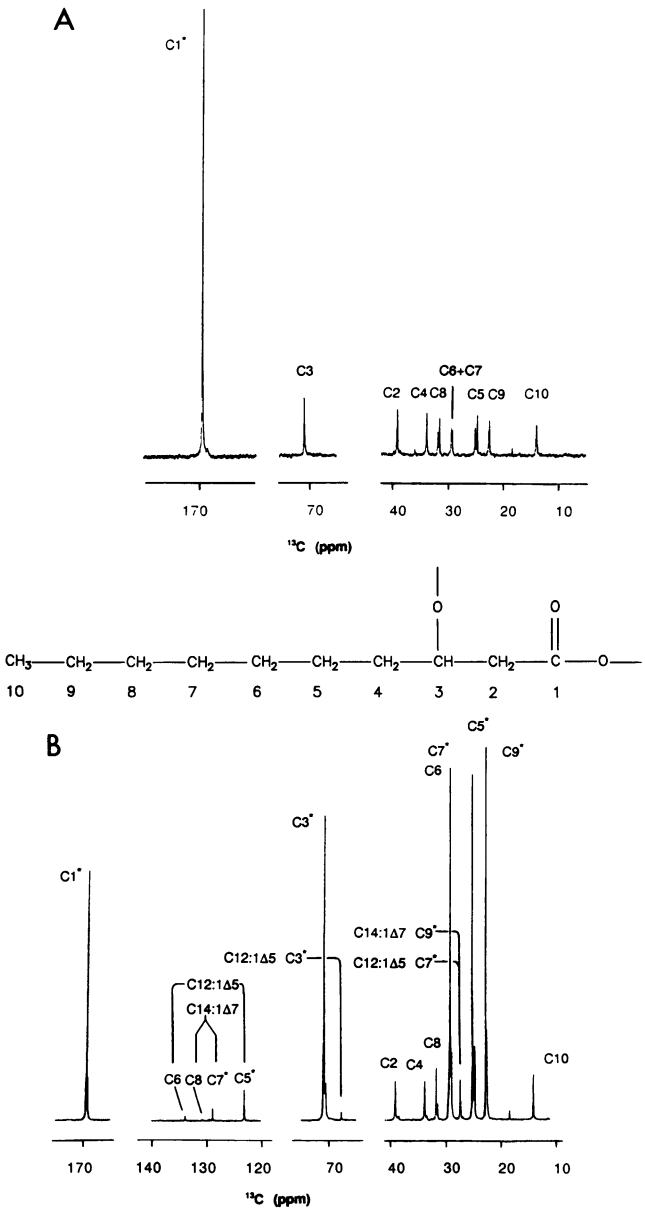

FIG. 1. ^{13}C NMR spectra of PHA isolated from *P. putida* KT2442 cultivated on [1-^{13}C]decanoate (A) and on glucose and [1-^{13}C]acetate (B). The assignments of the carbon atoms of the 3-hydroxydecanoate monomers (A) and of the major constituent 3-hydroxydecanoate (B) are indicated. ^{13}C-enriched carbon atoms are marked (*). The signals corresponding to carbon atoms in the double bonds of the unsaturated monomers are also assigned. The chemical shifts of all constituents can be found in Table 1.

Chemical ionization GC-MS analyses of ^{13}C-labeled PHA revealed that the 3-hydroxyhexanoate methyl esters contained one ^{13}C-enriched carbon atom. EI GC-MS data showed that the label was located in a fragment with an *m/e* of 103 corresponding to the carbon atoms C-1, C-2, and C-3 of the 3-hydroxy fatty acids. The ^{13}C NMR spectrum of PHA isolated from cells cultivated on [1-^{13}C]hexanoate is depicted in Fig. 3 together with the assignment. It can be seen that the intensity

TABLE 1. ^{13}C chemical shifts of PHA monomers isolated from *P. putida* KT2442 cultivated on glucose

Carbon	Chemical shift (ppm) of 3-hydroxyalkanoate monomers[a]						
	C_6	C_8	C_{10}	C_{12}	$C_{12:1}$	C_{14}	$C_{14:1}$
1	169.40	169.40	169.40	169.40	169.40	169.40	169.40
2	39.14	39.14	39.14	39.14	38.47	39.14	39.14
3	70.63	70.88	70.88	70.88	70.51	70.88	70.88
4	35.92	33.78	33.84	33.84	31.47	33.84	33.44
5	18.34	24.74	25.10	25.10	122.98	25.10	25.10
6	13.82	31.55	29.38	29.38	133.88	29.38	26.85
7		22.52	29.21	29.54	27.43	29.54	128.80
8		13.99	31.81	29.38	29.54	29.54	130.73
9			22.65	29.21	29.02	29.54	27.29
10			14.09	31.81	31.92	29.38	29.54
11				22.65	22.65	29.21	29.02
12				14.09	14.09	31.81	31.92
13						22.65	22.65
14						14.09	14.09

[a] Downfield from internal tetramethylsilane. C_6, 3-hydroxyhexanoate; C_8, 3-hydroxyoctanoate; C_{10}, 3-hydroxydecanoate; $C_{12:1}$, 3-hydroxy-*cis*-5-dodecenoate; C_{12}, 3-hydroxydodecanoate; $C_{14:1}$, 3-hydroxy-*cis*-7-tetradecenoate; C_{14}, 3-hydroxytetradecanoate.

of the signal assigned to the C-1 carbon atom is enhanced in comparison with the same signal in the ^{13}C NMR spectrum of PHA derived from unlabeled hexanoate. Comparing the intensities of the signals assigned to carbon atoms C-1 and C-2 in PHA synthesized from [1-^{13}C]hexanoate with those of the signals from unlabeled hexanoate, we estimate a 70-fold ^{13}C enrichment of carbon atom C-1 in the PHA derived from [1-^{13}C]hexanoate.

The EI and chemical ionization GC-MS analyses revealed that the 3-hydroxyoctanoate methyl esters derived from this PHA contain two ^{13}C-enriched carbon atoms and that these two carbon atoms are located in the fragment with an *m/e* of 103. From the ^{13}C NMR spectrum, it is evident that the odd-numbered carbon atoms are ^{13}C enriched, and therefore we conclude that ^{13}C enrichment in the 3-hydroxyoctanoate methyl esters has occurred at positions C-1 and C-3. In addition, a fragment with an *m/e* of 74 containing carbon atoms C-1 and C-2, which is the result of a McLafferty rearrangement (14) in 3-hydroxy fatty acid methyl esters, shows ^{13}C enrichment of only one carbon atom, thus excluding the possibility that carbon atom C-2 is ^{13}C enriched.

GC-MS analyses of the 3-hydroxydecanoate methyl esters show that three or more carbon positions are ^{13}C enriched. ^{13}C NMR analysis reveals a specific ^{13}C labeling pattern for all monomers with a carbon chain length of 10 or more (Fig. 3). Compared with the signals belonging to the even-numbered carbon atoms, the signals corresponding to the odd-numbered carbon atoms are enhanced, indicating a ^{13}C enrichment of the odd-numbered carbon atoms in these monomers.

TABLE 2. Composition of PHA isolated from *P. putida* KT2442 cultivated on decanoate, hexanoate, [1-^{13}C]decanoate, and [1-^{13}C]hexanoate[a]

Substrate	Relative amount of monomers in purified PHA (%, wt/wt)[b]						
	C_6	C_8	C_{10}	$C_{12:1}$	C_{12}	$C_{14:1}$	C_{14}
Decanoate	6	53	41				
[1-^{13}C]decanoate	5	52	43				
Hexanoate	72	14	10	1	1	1	1
[1-^{13}C]hexanoate	78	8	10	1	1	1	1

[a] Cells were cultivated as described in Materials and Methods and were harvested after 48 to 72 h. 20 mM hexanoate was added to the cultures in two portions of 10 mM with a time interval of 16 h to prevent toxicity.

[b] C_6, 3-hydroxyhexanoate; C_8, 3-hydroxyoctanoate; C_{10}, 3-hydroxydecanoate; $C_{12:1}$, 3-hydroxy-*cis*-5-dodecenoate; C_{12}, 3-hydroxydodecanoate; $C_{14:1}$, 3-hydroxy-*cis*-7-tetradecenoate; C_{14}, 3-hydroxytetradecanoate.

Inhibition of PHA synthesis. The fatty acid metabolic pathways, beta-oxidation and fatty acid synthesis, can be inhibited by acrylic acid and cerulenin, respectively. Acrylic acid inhibits the enzymes acyl-CoA synthase and 3-ketoacyl-CoA thiolase presumably via an interaction of acrylic acid with CoA (17). Cerulenin binds to the active sites of keto acyl synthases I and II (6, 12). We have determined the inhibitory concentrations of these compounds, and it was found that acrylic acid effectively inhibits beta-oxidation in *P. putida* KT2442 at 0.1 mg/ml. Cerulenin inhibits fatty acid synthesis in *P. putida* KT2442 at 0.3 mg/ml.

In order to study the effects of these inhibitors on the synthesis and composition of PHA, an exponentially growing culture of *P. putida* was harvested, washed, and resuspended in nitrogen-free medium containing either 20 g of glucose per liter or 10 mM octanoate. Under these conditions, PHA formation is observed within 24 h. The effects of the inhibition of fatty acid metabolism on PHA formation were examined by adding the appropriate amount of inhibitor to the cell suspensions.

In cell suspensions containing 10 mM octanoate as the substrate, no PHA formation was observed in the presence of 0.1 mg of acrylic acid per ml. Synthesis of PHA from octanoate, however, was affected by the presence of 0.3 mg of cerulenin per ml, but the composition of this PHA was identical to PHA synthesized from octanoate in the absence of the inhibitor (Table 3).

In cell suspensions with glucose as the substrate, PHA formation was inhibited by 0.3 mg of cerulenin per ml, whereas PHA formation was not affected by the presence of 0.1 mg of acrylic acid per ml. The composition of the PHA was not influenced by the presence of acrylic acid (Table 3).

DISCUSSION

PHA synthesis and de novo fatty acid synthesis. Previously, we proposed that during growth on non-PHA-related substrates, PHA precursors are generated by de novo fatty acid synthesis. In this study, the relation between PHA synthesis and the fatty acid metabolic pathways was further established by ^{13}C NMR analysis of PHAs purified from *P. putida* KT2442 cultivated on a combination of glucose and [1-^{13}C]acetate. From the GC analyses, it is clear that the addition of ^{13}C-labeled acetate to cultures does not result in aberrant PHA compositions, and so we conclude that this is a reliable way of studying the formation of PHA from non-PHA-related substrates.

The labeling pattern of PHA synthesized from [1-^{13}C]acetate can be understood from the reactions in the fatty acid biosynthetic pathway. First, acetyl-CoA molecules are carboxylated to malonyl-CoA and then converted to malonyl-ACP. Acetyl-CoA labeled at the C-1 position yields malonyl-ACP labeled at the C-1 position. Malonyl-ACP is coupled to the acyl chain with the release of CO_2, which results in the formation of acyl chains with ^{13}C label at odd-numbered carbon positions. The ^{13}C NMR spectra and the GC-MS data show that in PHA derived from [1-^{13}C]acetate the ^{13}C label is found at odd-

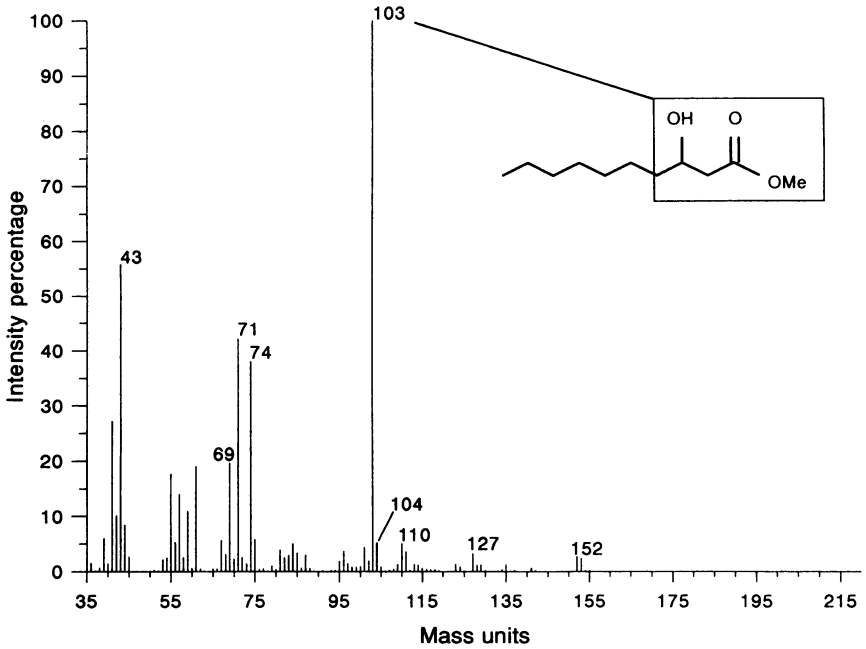

FIG. 2. EI GC-MS spectrum of 3-hydroxydecanoate methyl ester derived from PHA isolated from *P. putida* KT2442 cultivated on decanoate. The structure of the fragment with an *m/e* of 103, characteristic of 3-hydroxyalkanoate methyl esters, is shown. From the ratio of signals 103, 104, 105, and 106, the number of ^{13}C-enriched carbon atoms in the fragment can be calculated.

numbered carbon positions. In a similar way, the ^{13}C label is found at even-numbered positions when [2-^{13}C]acetate is used (5). These data confirm the role of fatty acid biosynthesis in PHA formation. However, the possibility that PHA precursors are derived (in part) from the beta-oxidation of newly synthesized fatty acids cannot be excluded. Therefore, we investigated the formation of PHA in the presence of the beta-oxidation inhibitor acrylic acid. At a concentration of 0.1 mg/ml, acrylic acid completely inhibited beta-oxidation, whereas the amount and the composition of PHAs were not affected (Table 3). From this, we conclude that the precursors for PHA polymerization can be directly and exclusively derived from the de novo fatty acid biosynthesis route.

PHA synthesis from 1-^{13}C-labeled fatty acids. PHA synthesized from [1-^{13}C]decanoate exhibits a specific ^{13}C enrichment of the C-1 carbon atoms. The GC-MS spectra revealed that the increase of label was present only in the 3-hydroxydecanoate methyl esters. No ^{13}C enrichment was found in the 3-hydroxyoctanoate and 3-hydroxyhexanoate methyl esters. These results support the hypothesis that intermediates for PHA polymerization are generated via beta-oxidation of fatty acids. The C_2 fragment containing the label is removed in the first cycle of beta-oxidation, yielding unlabeled intermediates with a carbon chain length of eight.

From ^{13}C NMR, GC, and GC-MS analyses of PHA formed by *P. putida* KT2442 growing on [1-^{13}C]hexanoate, it can be deduced that in this case PHA precursors are generated by three different routes. In Fig. 4, a schematic representation of the labeling patterns, as determined by ^{13}C NMR and GC-MS, is shown. Partial beta-oxidation of [1-^{13}C]hexanoate yields [1-^{13}C]3-hydroxyhexanoate monomers which account for approximately 72% of the PHA monomers. Degradation of [1-^{13}C]hexanoate via beta-oxidation yields three molecules of acetyl-CoA of which one is [1-^{13}C]acetyl-CoA. The presence of unsaturated monomers indicates that there is also PHA formation via de novo fatty acid synthesis. This is also clear from the labeling pattern of the medium-chain-length monomers (C_{10}, $C_{12:0}$, $C_{12:1}$, $C_{14:0}$, and $C_{14:1}$). The ^{13}C label is found at all odd-numbered carbon positions in these monomers. Apparently, the [1-^{13}C]acetyl-CoA which is formed in the degradation of [1-^{13}C]hexanoate is used as a substrate for the formation of PHA. The ratio in which these medium-chain-length monomers are present in PHA formed from hexanoate corresponds to the composition of PHA synthesized from carbohydrates. From these results, we estimate that 13% of the PHA monomers synthesized from hexanoate are derived from the de novo fatty acid biosynthetic pathway.

However, on the basis of the composition of PHA synthesized from carbohydrates, the de novo fatty acid biosynthetic route can account for only approximately 15% of the 3-hydroxyoctanoate monomers. The remaining 85% of the 3-hydroxyoctanoate monomers must be the result of another biosynthetic reaction. The ^{13}C NMR spectra and GC-MS data show that in the 3-hydroxyoctanoate monomers carbon atoms 1 and 3 are more frequently labeled. Therefore, we conclude that most of the 3-hydroxyoctanoate monomers in PHA synthesized from hexanoate are generated via elongation of hexanoate with, presumably, acetyl-CoA.

Relationship between fatty acid metabolism and PHA formation. In this report, we have provided further evidence that intermediates for PHA formation can be generated via de novo fatty acid synthesis, beta-oxidation, and elongation of fatty acids. An overview of the pathways and the structures of the intermediates formed in the synthesis of PHA from [1-^{13}C]hexanoate is shown in Fig. 4. The experiments with inhibitors of fatty acid metabolism have demonstrated that, depending on the nature of the substrate, precursors for PHA synthesis can be derived from either beta-oxidation or fatty acid biosynthesis. Both routes can operate independently and simultaneously.

The PHA polymerizing enzyme system appears to have a preference for monomers with 8 or 10 carbon atoms, while larger and smaller monomers are incorporated less efficiently

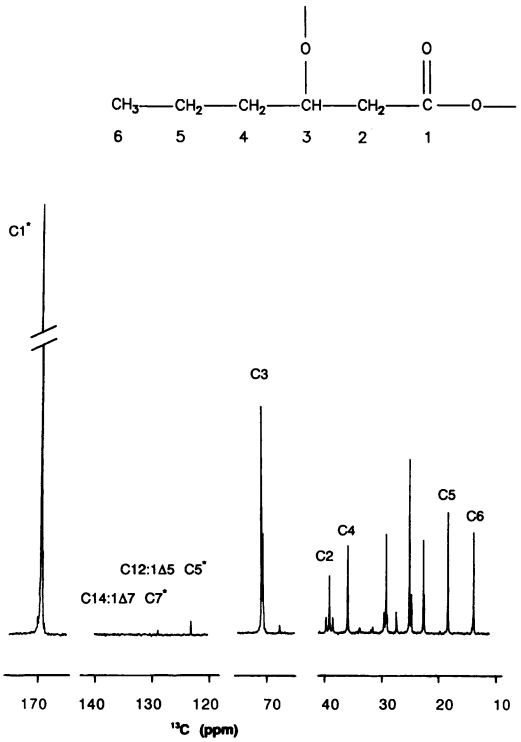

FIG. 3. ^{13}C NMR spectrum of PHA isolated from *P. putida* KT2442 cultivated on [1-^{13}C]hexanoate. The assignment of the signals corresponding to carbon atoms in the major compound 3-hydroxyhexanoate is indicated. The signals in the 120- to 140-ppm part of the spectrum correspond to carbon atoms in the unsaturated monomers 3-hydroxy-*cis*-5-dodecenoate and 3-hydroxy-*cis*-7-tetradecenoate. The unassigned signals in the 10- to 40-ppm part correspond to carbon atoms in the medium-chain-length monomers 3-hydroxyoctanoate, 3-hydroxydecanoate, 3-hydroxydodecanoate, and 3-hydroxytetradecanoate. The exact chemical shifts of the carbon atoms of these compounds are listed in Table 1.

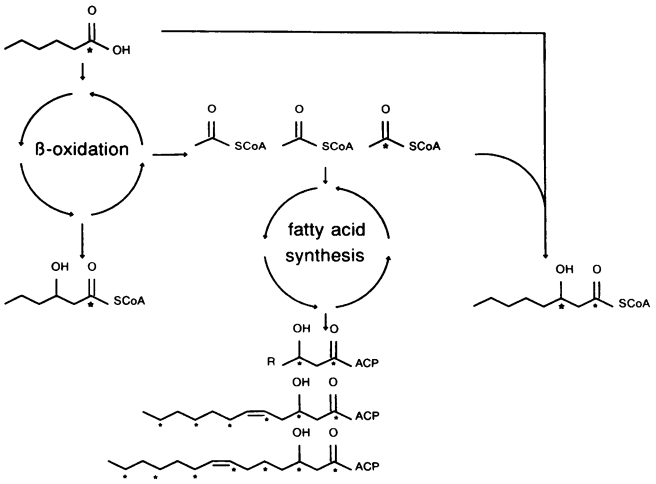

FIG. 4. Overview of reactions yielding precursors for PHA synthesis from hexanoate. 3-Hydroxyhexanoate monomers are synthesized via beta-oxidation, and the concomitantly generated acetyl-CoA molecules can be incorporated into PHA via de novo fatty acid biosynthesis. R represents the acyl groups of the saturated 3-hydroxyacyl-ACP intermediates. ^{13}C-labeled carbon atoms are indicated (∗), and less frequently labeled carbon atoms are also indicated (*). 3-Hydroxyoctanoate monomers can also be generated via elongation of hexanoate by an acetyl-CoA molecule.

(11). This might explain why, during growth on hexanoate, PHA monomers are synthesized by three different routes. Both elongation and de novo fatty acid synthesis result in the generation of the more preferable C_8 and C_{10} monomers. The presence of PHA monomers with a larger chain length than the substrate has been observed before (2, 7). Haywood et al. (8) have suggested that these monomers are formed via a condensation reaction of acyl-CoA molecules with acetyl-CoA catalyzed by the beta-oxidation enzyme 3-ketothiolase. It cannot be ruled out, however, that elongation of exogenous fatty acids in *P. putida* proceeds via de novo fatty acid biosynthesis. In *P. putida*, 3-hydroxydecanoate is the major constituent of PHA synthesized via de novo fatty acid biosynthesis. If elongation of hexanoate in *P. putida* were the result of de novo fatty acid biosynthesis, we would expect 3-hydroxydecanoate to be the predominant product and only a minor amount of 3-hydroxyoctanoate to be present. Since this is not the case, we conclude that elongation of hexanoate via a reverse action of the 3-ketothiolase is more likely than elongation via de novo fatty acid biosynthesis.

TABLE 3. Inhibition of *P. putida* KT2442 fatty acid metabolic routes and effects on PHA synthesis[a]

Substrate	% PHA[b]	Relative amount of monomers in PHA (%, wt/wt)[c]						
		C_6	C_8	C_{10}	$C_{12:1}$	C_{12}	$C_{14:1}$	C_{14}
1.5% glucose	8.5	1	11	66	11	8	2	1
1.5 glucose + acrylic acid	8.3	1	12	65	12	8	1	1
1.5% glucose + cerulenin	—[d]	—	—	—	—	—	—	—
10 mM octanoate	22.3	6	92	2	—	—	—	—
10 mM octanoate + acrylic acid	—	—	—	—	—	—	—	—
10 mM octanoate + cerulenin	7.0	5	94	1	—	—	—	—

[a] Cell suspensions of *P. putida* KT2442 were incubated with substrates and inhibitors as described in Materials and Methods.
[b] Percent PHA was determined by GC analysis and calculated as a percentage of cell dry weight.
[c] C_6, 3-hydroxyhexanoate; C_8, 3-hydroxyoctanoate; C_{10}, 3-hydroxydecanoate; $C_{12:1}$, 3-hydroxy-*cis*-5-dodecenoate; C_{12}, 3-hydroxydodecanoate; $C_{14:1}$, 3-hydroxy-*cis*-7-tetradecenoate; C_{14}, 3-hydroxytetradecanoate.
[d] —, not detected (<0.1%).

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