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Determination of Sequence Distributions in Bacterial Copolyesters Containing Higher Alkyl and Alkenyl Pendant Groups

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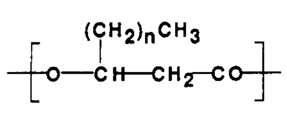
ABSTRACT: The sequence distributions of microbial copolyesters produced by *Pseudomonas oleovorans* grown with (a) nonanoic acid (NA), (b) an equimolar mixture of NA and octanoic acid (OA), or (c) 2:1 mixtures of 10-undecenoic acid (UD) with either NA or OA were determined by analyzing the oligomers prepared by partial pyrolysis of these copolyesters using fast atom bombardment mass spectrometry. Oligomers up to tetramers were identified, and the amounts of these oligomers were used to calculate repeating unit compositions and sequence distributions. The sequence distributions in these copolyesters followed Bernoullian statistics, indicating that they were random copolymers. No ester exchange occurred during the pyrolysis.

Introduction

Poly(β-hydroxyalkanoates) (PHAs) are optically active polyesters that are produced as intracellular energy and carbon storage material by a wide variety of bacteria.[1] These biopolymers have the following general structure:



$$\left[\, O-\overset{(CH_2)_nCH_3}{\overset{|}{C}H}-CH_2-CO \,\right]$$

in which n can vary from 0 to at least 9 depending on the carbon substrate used for growth and the microorganism. In many cases, the bacteria produce copolyesters, and the copolymers containing 3-hydroxybutyrate ($n = 0$) and 3-hydroxyvalerate ($n = 1$) are often found in nature.[2-7] The compositions of these copolymers are readily determined by ^{1}H NMR,[2-5] and the sequence distributions of their repeating units can be readily determined from their ^{13}C NMR spectra.[3,5]

In our investigation of bacterial polyesters we have been particularly interested in the polyesters produced by *P. oleovorans* when grown aerobically with either n-alkanes[9] or alkanoic acids.[10-12] In general, the PHAs produced by *P. oleovorans* have relatively long alkyl pendant groups that can vary in size from $n = 2$ to $n = 9$ and probably higher depending on the growth substrate. Furthermore, PHAs containing both 3-hydroxyalkanoate and unsaturated 3-hydroxyalkenoate units can be produced by *P. oleovorans* grown with 1-alkenes[9,10] or alkenoic acids.[13,21]

The compositions and sequence distributions of the repeating units in these PHAs with long alkyl pendant group cannot be determined by NMR spectrometry because the chemical shifts of the carbon atoms in the units comprised of β-hydroxyhexanoate groups or higher alkanoates are essentially identical, and the patterns of their proton NMR spectra are too complicated to be used for such determination.[11,12] However, the sequence distribution of the repeating units can be determined by analyzing oligomers produced from the random decomposition of these copolymers.

We have previously reported on the use of fast atom bombardment (FAB) mass spectrometry to determine the repeating unit composition and the sequence distribution

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Table I
Composition of the PHA Samples Obtained by Gas Chromatography and FAB Mass Spectrometry

			repeating units present,[a] mol %									
sample	carbon source		HV	HC	HH_{ol}	HH	HO	HN_{ol}	HN	HD	HU_{ol}	HU
1	nonanoate	GC	2			26			70			2
		MS	2			31			65			2
2	1/1 octanoate/nonanoate	GC	1	5		16	35		40	2		1
		MS	2	6		16	42		28	4		2
3	2/1 octanoate/10-undecenoate	GC		4	2		54	23			12	
		MS		6	4		60	20			10	
4	2/1 nonanoate/10-undecenoate	GC			3	12		26	45		12	
		MS			7	20		25	38		10	

[a] See text for abbreviations used.

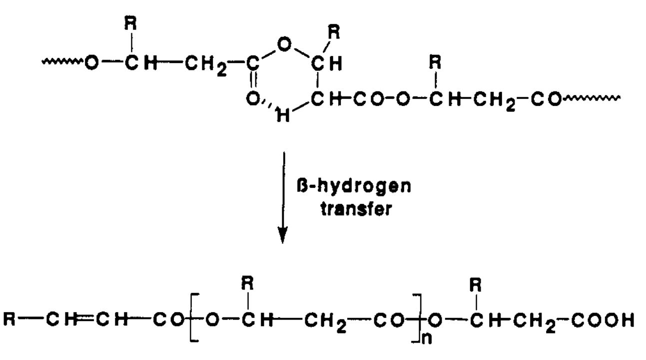
of repeating units in copolymers with $n = 0$ and 1[8] and in higher PHAs produced by *P. oleovorans* grown with *n*-alkanoic acids.[14] In those determinations, the oligomers were obtained by partial methanolysis of the copolyesters.

The application of mass spectrometry is not limited by the size of the repeating units, and large oligomers, such as tetramer or larger, can be identified and used for the determination of composition and sequence distributions, but partial methanolysis is time-consuming, so a new method for the preparation of oligomers was developed based on the ester pyrolysis reaction. Partial pyrolysis proved to be a fast and accurate method for the microstructure analysis of the PHAs with lower alkyl pendant groups.[15]

In the present study the microstructures of PHAs produced by *P. oleovorans* grown with nonanoic acid (NA) (sample 1), an equimolar mixture of NA and octanoic acid (OA) (sample 2), or 2:1 mixtures of 10-undecenoic acid (UD) with either OA (sample 3) or NA (sample 4) were determined by FAB-MS analysis of their partial pyrolysis products. The possible occurrence of ester exchange reactions during the pyrolysis was also examined. The repeating units in these PHAs are designated by the following abbreviations: HC, 3-hydroxycaproate ($R = C_3H_7$); HH_{ol}, 3-hydroxyheptenoate ($R = C_4H_7$); HH, 3-hydroxyheptanoate ($R = C_4H_9$); HO, 3-hydroxyoctanoate ($R = C_5H_{11}$); HN_{ol}, 3-hydroxynonenoate ($R = C_6H_{11}$); HN, 3-hydroxynonanoate ($R = C_6H_{13}$); HD, 3-hydroxydecanoate ($R = C_7H_{15}$); HU_{ol}, 3-hydroxyundecenoate ($R = C_8H_{15}$); HU, 3-hydroxyundecanoate ($R = C_8H_{17}$).

Results and Discussion

It is well-known[15-18] that the thermal decomposition, or ester pyrolysis, of PHAs occurs by a β-hydrogen elimination reaction with formation of oligomers having carboxyl and olefin end groups as shown below:



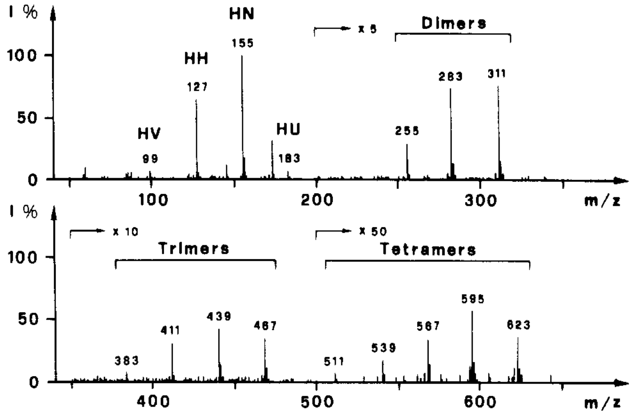


Figure 1. Negative ion FAB mass spectrum of the TG residue from the partial pyrolysis of sample 1.

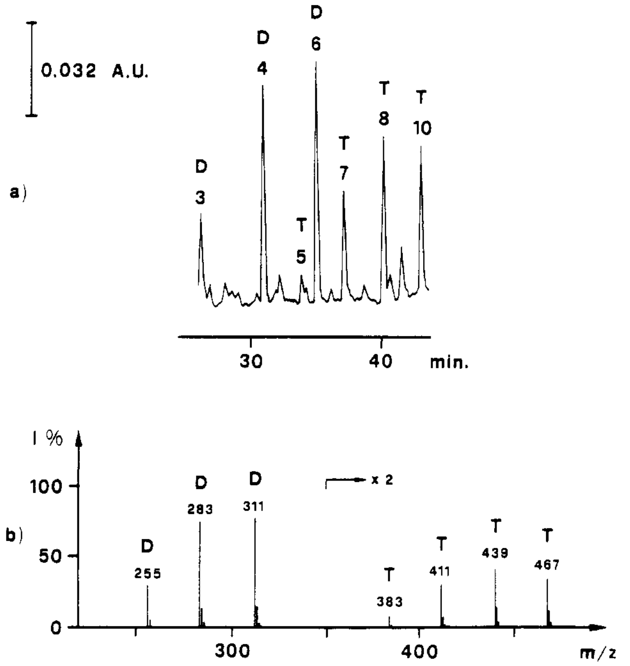


Figure 2. HPLC separation of dimers (D) and trimers (T) of partial methanolysis products (a) and negative ion FAB mass spectrum of dimers and trimers of partial pyrolysis products (b) of sample 1.

It has been shown that the molecular weight changes which occur during this type of thermal degradation follow the model of random chain scission of the ester group, and the rate of scission is not dependent on either the type of substituent R or the composition of the copolyesters.[18]

The residues of the partial pyrolysis of these polyesters consist of oligomers of various sizes having the end groups shown above. Because these carboxylic acids lose a proton easily when they are ionized by the FAB source, the spectra were recorded in the negative ion FAB mode to reveal their presence as carboxylate ions. The negative ion FAB mass spectrum of the pyrolysate of sample 1 (Table I) at

Table II
Experimental[a] and Calculated[b] Relative Amounts of the Partial Pyrolysis Products[c] from TG and FAB-MS Analyses of Sample 1

oligomers[c]	m/z (M − H)$^-$	obsd from TG FAB-MS[a]	calcd[b] for HV/HH/HN/HU mole ratio 2/26/70/2	2/31/65/2	0/40/60/0
dimers					
$(HH)_2$, (HV)(HN)	255	16	10	13	16
(HH)(HN), (HV)(HU)	283	41	38	42	48
$(HN)_2$, (HH)(HU)	311	43	52	45	36
AF[d]			0.172	0.059	0.159
trimers					
C_{21}[e]	383	7	4	6	6
C_{23}	411	26	18	22	29
C_{25}	439	37	40	41	43
C_{27}	467	30	38	31	22
AF[d]			0.208	0.104	0.185
tetramers					
C_{28}	511	4	2	3	2
C_{30}	539	11	8	11	16
C_{32}	567	23	24	26	35
C_{34}	595	39	38	38	34
C_{36}	623	23	28	22	13
AF[d]			0.118	0.066	0.326

[a] Relative intensities of (M − H)$^-$ ions of the pyrolysis products of the FAB mass spectrum. [b] Relative intensities of pyrolysis products, calculated by the equation in the text for three copolymer compositions. [c] The pyrolysis products contain variable amounts of the following monomer units (see text for abbreviations used): HV (m/z 99), HH (m/z 127), HN (m/z 155), HU (m/z 183). [d] Agreement factor.[15,20] [e] All possible trimers containing 21 carbon atoms that can be obtained from monomers are indicated as C_{21} trimers (i.e., $(HH)_3$, (HV)(HH)(HN), ...). The identities of the remaining C_n oligomers (reported in this table) can be deduced in the same way.

Table III
Experimental[a] and Calculated[b] Relative Amounts of the Partial Pyrolysis Products[c] from TG and FAB-MS Analyses of Sample 2

oligomers[c]	m/z (M − H)$^-$	obsd from TG FAB-MS[a]	calcd[b] for HV/HC/HH/HO/HN/HD/HU mole ratio 1/5/16/35/40/2/1	2/6/16/42/28/4/2	1/5/16/55/20/2/1
dimers					
(HV)(HO), (HC)(HH)	241	4	2	4	2
(HV)(HN), (HC)(HO), $(HH)_2$	255	9	7	9	8
(HV)(HD), (HC)(HN), (HH)(HO)	269	17	15	18	20
(HV)(HU), (HC)(HD), (HH)(HN), $(HO)_2$	283	29	26	28	38
(HC)(HU), (HH)(HD), (HO)(HN)	297	27	29	26	23
(HH)(HU), (HO)(HD), $(HN)_2$	311	12	18	12	7
(HN)(HD), (HO)(HU)	325	2	3	3	2
AF[d]			0.171	0.044	0.234
trimers					
C_{21}[e]	383	5	3	5	4
C_{22}	397	9	8	10	11
C_{23}	411	15	14	17	20
C_{24}	425	26	21	23	30
C_{25}	439	23	25	23	22
C_{26}	453	14	20	15	10
C_{27}	467	8	9	7	3
AF[d]			0.199	0.096	0.208
tetramers					
C_{30}	539	13	8	12	13
C_{31}	553	15	14	17	21
C_{32}	567	23	20	22	27
C_{33}	581	23	23	22	22
C_{34}	595	15	21	17	12
C_{35}	609	11	14	10	5
AF			0.209	0.082	0.222

[a] Relative intensities of (M − H)$^-$ ions of the pyrolysis products of the FAB mass spectrum. [b] Relative intensities of pyrolysis products, calculated by the equation in the text for three copolymer compositions. [c] The pyrolysis products contain variable amounts of the following monomer units (see text for abbreviations used): HV (m/z 99), HC (m/z 113), HH (m/z 127), HO (m/z 141), HN (m/z 155), HD (m/z 169), HU (m/z 183). [d] Agreement factor.[15,20] [e] All possible trimers containing 21 carbon atoms that can be obtained from monomers are indicated as C_{21} trimers (i.e., $(HH)_3$, $(HV)_2(HU)$, $(HC)_2(HN)$, ...). The identity of the remaining C_n oligomers (reported in this table) can be deduced in the same way.

20 wt % loss is shown in Figure 1. The spectrum essentially consists of the (M − H)$^-$ pseudomolecular ions corresponding to the oligomers of the structure shown in the reaction above (the matrix peaks have been subtracted). The oligomers identified in this manner are listed in Table II. The spectrum in Figure 1 shows little evidence of the fragmentation of molecular ions. Therefore, the intensities of the molecular ions can be used for quantitative analysis to estimate the copolymer compositions and sequence distributions.

The HPLC chromatogram for the dimers and trimers prepared by partial methanolysis of sample 1 is shown in Figure 2a,[14] and the corresponding FAB mass spectrum of the oligomers prepared by partial pyrolysis is shown in Figure 2b. The intensities of the peaks in the HPLC chromatogram and in the FAB mass spectrum correspond to

Table IV
Experimental[a] and Calculated[b] Relative Amounts of the Partial Pyrolysis Products[c] from TG and FAB-MS Analyses of Sample 3

oligomers[c]	m/z (M − H)$^-$	obsd from TG FAB-MS[a]	calcd[b] for HC/HH$_{ol}$/HO/HN$_{ol}$/HU$_{ol}$ mole ratio 4/2/54/23/12	6/4/60/20/10	5/3/70/10/12
dimers					
(HC)(HO)	255	7	5	7	7
(HC)(HN$_{ol}$), (HH$_{ol}$)(HO)	267	7	6	7	5
(HO)$_2$	283	37	32	37	50
(HC)(HU$_{ol}$), (HO)(HN$_{ol}$)	295	26	28	26	15
(HN$_{ol}$)$_2$, (HH$_{ol}$)(HU$_{ol}$)	307	6	6	5	2
(HO)(HU$_{ol}$)	323	12	15	13	17
(HN$_{ol}$)(HU$_{ol}$)	335	4	6	4	3
(HU$_{ol}$)$_2$	363	1	2	1	1
			AF[d] 0.149	0.029	0.329
trimers					
C$_{23}$[e]	409	10	6	10	8
C$_{24}$	425	24	21	26	40
C$_{25}$	437	25	29	28	20
C$_{26}$	449	12	14	12	5
C$_{27}$	465	15	14	13	20
C$_{28}$	477	10	13	9	6
C$_{29}$	489	4	3	2	1
			AF[d] 0.172	0.106	0.358
tetramers					
C$_{31}$	551	10	8	12	9
C$_{32}$	567	18	14	18	31
C$_{33}$	579	29	27	28	22
C$_{34}$	591	19	20	17	8
C$_{35}$	607	12	13	12	21
C$_{36}$	619	12	18	13	9
			AF[d] 0.182	0.073	0.451

[a] Relative intensities of (M − H)$^-$ ions of the pyrolysis products of the FAB mass spectrum. [b] Relative intensities of pyrolysis products, calculated by the equation in the text for three copolymer compositions. [c] The pyrolysis products contain variable amounts of the following monomer units (see text for abbreviations used): HC (m/z 113), HH$_{ol}$ (m/z 125), HO (m/z 141), HN$_{ol}$ (m/z 153), HU$_{ol}$ (m/z 181). [d] Agreement factor.[15,20] [e] All possible trimers containing 23 carbon atoms that can be obtained from monomers are indicated as C$_{23}$ trimers (i.e., (HH$_{ol}$)(HO)$_2$, (HC)(HO)(HN$_{ol}$), ...). The identities of the remaining C$_n$ oligomers (reported in this table) can be deduced in the same way.

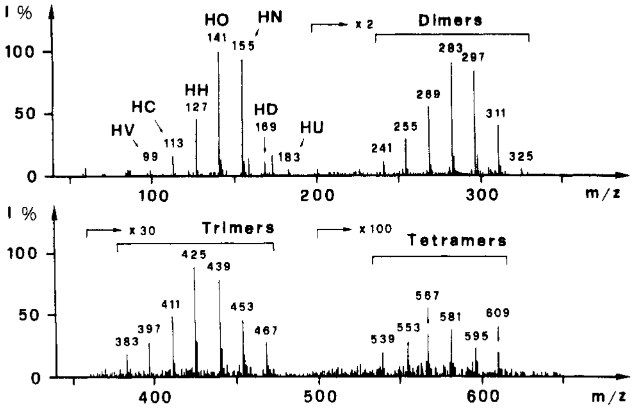


Figure 3. Negative ion FAB mass spectrum of the TG residue from the partial pyrolysis of sample 2.

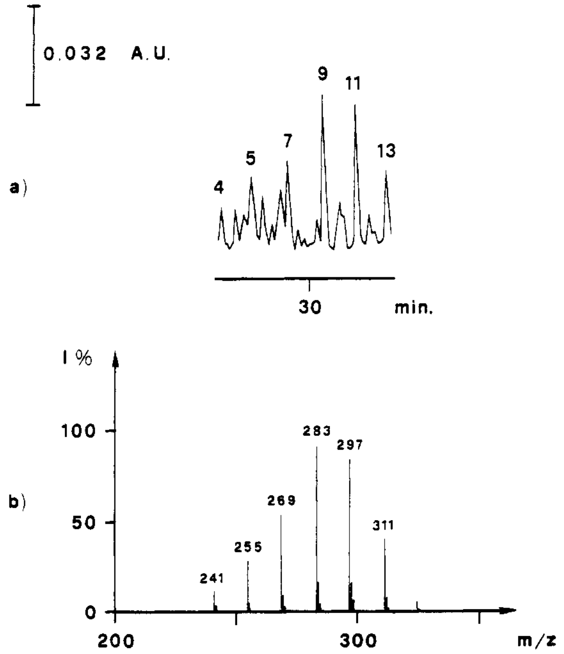


Figure 4. HPLC separation of dimers of partial methanolysis products (a) and negative ion FAB mass spectrum of dimers of partial pyrolysis products (b) of sample 2.

one another very closely. If it can be assumed that the response factors of the oligomers to FAB-MS are constant, the normalized intensities of the peaks corresponding to dimers, trimers, and tetramers should correspond to the copolymer composition. The monomer compositions can be determined on the assumption of a random distribution of repeating units in the copolymer. The probability of finding a given (HV)$_x$(HC)$_y$...(HU)$_z$ sequence, $P_{x,y,\ldots,z}$, assuming Bernoullian (random) statistics for each oligomer, is given by the Leibnitz formula[19] as follows:

$$P_{x,y,\ldots,z} = \frac{x + y + \ldots + z}{x!y!\ldots z!} P^x_{\mathrm{HV}} P^y_{\mathrm{HC}} \ldots P^z_{\mathrm{HU}}$$

in which P_{HV}, P_{HC}, ..., P_{HU} are the molar fractions of HV, HC, ..., HU units in the copolymer. The polynomial coefficient in this equation is the number of possible sequence arrangements of the (HV)$_x$(HC)$_y$...(HU)$_z$ oligomers. It is possible to find a set of $P^x_{\mathrm{HV}}P^y_{\mathrm{HC}}\ldots P^z_{\mathrm{HU}}$ which gives an oligomer distribution closest to that experimentally found, and this composition gives the lowest agreement factor (AF) described in our previous studies.[19] The calculations have been performed by a computer program named MACO 3 (Mass Analysis of Copolymers).[19] This program is an upgraded version of MACO 1.[15] MACO 1 is

Table V
Experimental[a] and Calculated[b] Relative Amounts of the Partial Pyrolysis Products[c] from TG and FAB-MS Analyses of Sample 4

oligomers^c	m/z (M – H)$^-$	obsd from TG FAB-MS	calcd^b for HH$_{ol}$/HH/HN$_{ol}$/HN/HU$_{ol}$		
			3/12/26/45/12	7/20/25/38/10	5/12/45/26/12
dimers					
(HH$_{ol}$)$_2$	251	1		1	
(HH$_{ol}$)(HH)	253	4	1	3	1
(HH)$_2$	255	4	2	4	2
(HH$_{ol}$)(HN$_{ol}$)	279	4	2	3	5
(HH)(HN$_{ol}$), (HH$_{ol}$)(NN)	281	13	9	15	13
(HH)(HN)	283	13	11	15	6
(HN$_{ol}$)$_2$, (HH$_{ol}$)(HU$_{ol}$)	307	7	8	8	22
(HN$_{ol}$)(HN), (HH)(HU$_{ol}$)	309	21	27	23	26
(HN)$_2$	311	17	21	14	7
(HN$_{ol}$)(HU$_{ol}$)	335	5	7	5	11
(HN)(HU$_{ol}$)	337	9	11	8	6
(HU$_{ol}$)$_2$	363	2	1	1	1
		AF^d	0.249	0.138	0.536
trimers					
C$_{23}$^{e}	409	7	2	7	3
	411	2	2	5	1
C$_{25}$	435	10	5	10	12
	437	13	12	17	11
	439	8	8	9	2
C$_{27}$	461	3	2	3	12
	463	12	14	13	22
	465	17	21	17	12
	467	9	10	6	2
C$_{29}$	489	4	3	3	8
	491	4	10	3	10
	493	7	8	5	2
C$_{31}$	517	1	1	1	2
	519	3	2	1	1
		AF^d	0.311	0.205	0.569
tetramers					
C$_{32}$	563	10	2	8	5
	565	8	4	10	4
C$_{34}$	591	9	10	15	16
	593	12	14	16	8
C$_{36}$	617	10	8	8	21
	619	17	19	16	19
	621	12	18	12	6
	623	8	6	3	1
C$_{38}$	645	5	7	5	13
	647	9	12	7	7
		AF^d	0.336	0.281	0.520

[a] Relative intensities of (M – H)$^-$ ions of the pyrolysis products of the FAB mass spectrum. [b] Relative intensities of pyrolysis products, calculated by the equation in the text for three copolymer compositions. [c] The pyrolysis products contain variable amounts of the following monomer units (see text for abbreviations used): HH$_{ol}$ (m/z 125), HH (m/z 127), HN$_{ol}$ (m/z 153), HN (m/z 155), HU$_{ol}$ (m/z 181). [d] Agreement factor.[15],[20] [e] All possible trimers containing 23 carbon atoms that can be obtained from monomers are indicated as C$_{23}$ trimers (i.e., (HH)$_2$(HN$_{ol}$), (HH$_{ol}$)(HH)(HN)). The identities of the remaining C$_n$ oligomers (reported in this table) can be deduced in the same way.

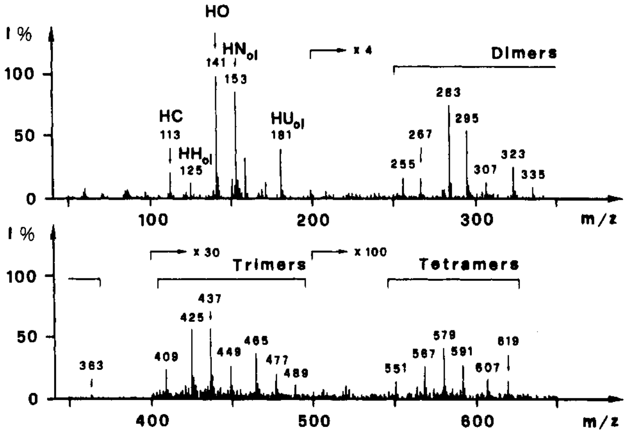


Figure 5. Negative ion FAB mass spectrum of the TG residue from the partial pyrolysis of sample 3.

applicable only to two-component copolymers, while MACO 3 is applicable to multicomponent copolymers.[19]

The calculated results for sample 1 are listed in Table II. The lowest AF was obtained for the repeating unit composition of 2/31/65/2 regardless of the oligomers investigated. This result is identical to that obtained in our previous report in which oligomers were prepared by partial methanolysis.[14]

In Figure 3 is shown the FAB mass spectrum of sample 2, and the identified oligomers are listed in Table III. The number of peaks in the mass spectrum of the oligomers from sample 2 was higher than that in the mass spectrum of the oligomers from sample 1 because there are more types of repeating units in sample 2 as shown in Table I. The HPLC chromatogram for dimers prepared by partial methanolysis of sample 2[14] and the corresponding pyrolysis–FAB mass spectrum are shown in Figure 4. The intensities in Figure 4 correspond to one another very closely.

The intensities of peaks in Figure 3 were compared with those calculated according to the equation above with results given in Table III. An HV/HC/HH/HO/HN/HD/HU composition of 2/6/16/42/28/4/2 gave the lowest AF. This composition is identical to that obtained from HPLC analysis.[14,19] These results confirm that sample 2 is a

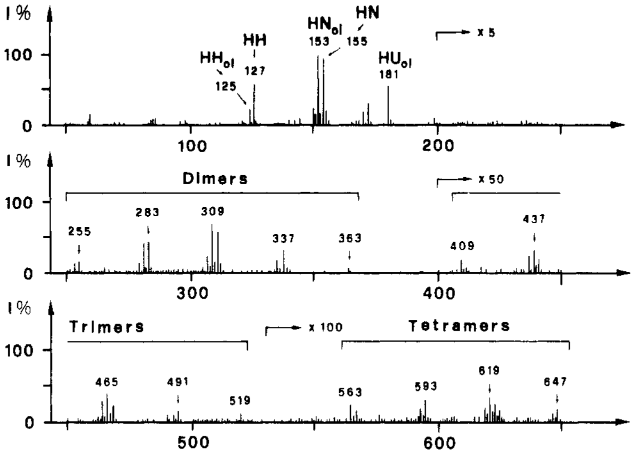


Figure 6. Negative ion FAB mass spectrum of the TG residue from the partial pyrolysis of sample 4.

random copolymer produced by *P. oleovorans* grown with NA/OA mixtures.

In Figures 5 and 6 are shown the FAB mass spectra of oligomers prepared by partial pyrolysis of samples 3 and 4, respectively. For these two samples, a peak at *m/z* 153 arising from the liquid matrix altered the intensity of the peak for the olefinic nonanoate units, HN_{ol} *m/z* 153. The intensities of oligomers determined by mass spectra and the calculated oligomer intensities for different monomer compositions are listed in Tables IV and V for samples 3 and 4, respectively. The lowest AF was obtained for an $HC/HH_{ol}/HO/HN_{ol}/HU_{ol}$ composition of 6/4/60/20/10 and an $HH_{ol}/HH/HN_{ol}/HU_{ol}$ composition of 7/20/25/38/10 for samples 3 and 4, respectively. In both cases the MS results did not vary far from those obtained by GC, so these results confirm that samples 3 and 4 are also random copolymers.

An experiment was performed to demonstrate that thermal degradation occurs without ester exchange reactions during the pyrolysis. A mixture of samples 1 and 3 (50/50 w/w), which had no monomeric units in common (Table I, Figures 1 and 5), was heated in the TG apparatus under a nitrogen atmosphere at 170 °C, which is below the thermal degradation temperatures and above the melting points, for 6 h and then at 220 °C until 20% weight was lost. If ester exchange had occurred, oligomers containing components from both samples 1 and 3 should have been found, while the absence of such oligomers would demonstrate that this reaction did not occur. All of the peaks corresponding to the components belonging to samples 1 (labeled a) and 3 (labeled b) were identified by comparing the mass spectrum of oligomers from the mixture (Figure 7) with those of oligomers from samples 1 and 3 (Figures 1 and 5). It can be concluded, therefore, that no new oligomers were formed as a result of ester exchange. That is, considering only dimers, there were no peaks of the following types, which would be formed by ester exchange, in Figure 7: (HC)(HH), (HV)(HO), *m/z* 241; $(HH)_2$, (HC)(HO), (HV)(HN), *m/z* 253; (HC)(HN), (HH)(HO), *m/z* 269; $(HH_{ol})(HN)$, $(HH)(HN_{ol})$, $(HV)(HU_{ol})$, *m/z* 281; (HO)(HN), (HC)(HU), *m/z* 297; $(HH)(HU_{ol})$, $(HN_{ol})(HN)$, $(HH_{ol})(HU)$, *m/z* 309; (HO)(HU) *m/z* 325; $(HN)(HU_{ol})$, $(HN_{ol})(HU)$, *m/z* 337.

These results show that the rate of the β-hydrogen-transfer reaction was much higher than that of an ester exchange reaction under the pyrolysis conditions used in this study.

Experimental Section

Polyester Production. The polyesters analyzed in this study were produced by *P. oleovorans* grown with either NA, an equimolar mixture of NA and OA, or 2:1 mixtures of UD and either OA or NA. The growth conditions are described in our previous report.[14] The total initial concentration of carboxylic acids was 10 mM, and the cells were harvested when the growth stopped. PHAs were extracted from the dry cells with hot chloroform under a nitrogen atmosphere.

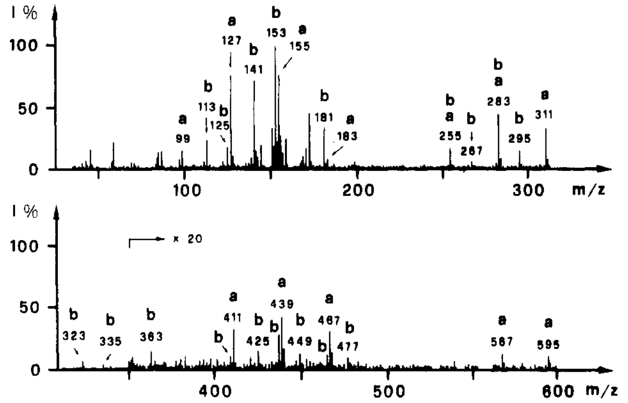


Figure 7. Negative ion FAB mass spectrum of partial pyrolysis products of a mixture (50% w/w) of sample 1 (a) and sample 3 (b).

GC Assay. The repeating unit compositions were determined by gas chromatography analysis of the acid-catalyzed methanolysis products of the polymers following the procedure described in our previous study.[14]

Molecular Weights. Molecular weights were determined by gel permeation chromatography (GPC) as described in our previous study.[14] The number-average molecular weights of all these PHAs were between 50 000 and 60 000, and the polydispersity indexes were between 1.8 and 2.1.

Partial Pyrolysis. Partial pyrolyses of microbial polyesters were performed with a Perkin-Elmer TGS/2 thermogravimetric (TG) apparatus under a nitrogen atmosphere (60 mL/min) at a heating rate of 10 °C/min. The temperature was increased up to the point at which the weight losses were 2, 5, 10, 20, 40, and 60%. After cooling rapidly to room temperature (100 °C/min), the residue was recovered. This residue was completely soluble in chloroform.

Negative Ion FAB Mass Spectra. A double-focusing Kratos MS 50 S mass spectrometer equipped with the standard FAB source and a DS 90 data system was used to obtain mass spectra. The FAB gun (Ion Tech) was operated in negative mode with a 7–8-keV xenon beam. The instrument was scanned from *m/z* 2200 to *m/z* 60, with a scan rate of 10 s/decade. The accelerating voltage was 6–8 kV. Cesium and rubidium iodides (50:50 by weight) were used for computer calibration. The resolution was approximately 3000. Samples obtained from partial pyrolysis of the polymers in TG experiments were placed on the copper target of the direct insertion probe and mixed with 3-nitrobenzyl alcohol as a matrix to dissolve the oligomers. Peak intensity values shown in the mass spectra or in the tables in this report represent the average of results from three separate experiments. The results obtained from samples pyrolyzed to various extents of weight loss did not vary significantly. Deviation in the values of the relative peak intensities were below 3% for dimers and trimers and below 7% for tetramers. The experimental peak intensities corresponding to monomers were excluded from the calculations, because the volatility of the monomers may be different from those of the others, so the measured amounts may not reflect exact composition of the copolymer.[15]

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References and Notes

(1) Dawes, E. A.; Senior, P. J. *J. Adv. Microbiol. Physiol.* **1973**, *10*, 135.

(2) Bloembergen, S.; Holden, D. A.; Hamer, G. K.; Bluhm, T. L.; Marchessault, R. H. *Macromolecules* **1986**, *19*, 2865; **1989**, *22*, 1663.

(3) Bluhm, T. L.; Hamer, G. K.; Marchessault, R. H.; Fyfe, C. A.; Veregin, R. P. *Macromolecules* **1986**, *19*, 2871.

(4) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1986**, *19*, 2860.

(5) Kamiya, N.; Yamamoto, Y.; Inoue, Y.; Chujo, R.; Doi, Y. *Macromolecules* **1989**, *22*, 1672; **1989**, *22*, 3800.

(6) King, P. P. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 2.

(7) Holmes, P. A. *Phys. Technol.* **1985**, *16*, 32.

(8) Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Montaudo, G. *Macromolecules* **1989**, *22*, 2107.

(9) Lageveen, R. G.; Huisman, G. W.; Presuting, H.; Ketelaar, P.; Eggink, G.; Witholt, G. *Appl. Environ. Microbiol.* **1988**, *54*, 2924.

(10) Preusting, H.; Nijenhuis, A.; Witholt, B. *Macromolecules* **1990**, *23*, 4220.

(11) Gross, R. A.; DeMello, C.; Lenz, R. W.; Brandl, H.; Fuller, R. C. *Macromolecules* **1989**, *22*, 1106.

(12) Brandl, H.; Gross, R. A.; Lenz, R. W.; Fuller, R. C. *Appl. Environ. Microbiol.* **1988**, *54*, 1977.

(13) Fritzche, K.; Lenz, R. W.; Fuller, R. C. *Int. J. Biol. Macromol.* **1990**, *12*, 85.

(14) Ballistreri, A.; Montaudo, G.; Impallomeni, G.; Lenz, R. W.; Kim, Y. B.; Fuller, R. C. *Macromolecules* **1990**, *23*, 5059.

(15) Ballistreri, A.; Montaudo, G.; Garozzo, D.; Giuffrida, M.; Montaudo, M. S. *Macromolecules* **1991**, *24*, 1231.

(16) Morikawa, H.; Marchessault, R. H. *Can. J. Chem.* **1981**, *59*, 2306.

(17) Grassie, N.; Murray, E. J.; Holmes, P. A. *Polym. Degrad. Stab.* **1984**, *6*, 127.

(18) Kunioka, M.; Doi, Y. *Macromolecules* **1990**, *23*, 1933.

(19) Montaudo, M. S.; Ballistreri, A.; Montaudo, G. *Macromolecules* **1991**, *24*, 5051.

(20) Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

(21) Kim, Y. B.; Lenz, R. C.; Fuller, R. C., manuscript in preparation.

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