Macromolecules **1988**, *21*, 2722–2727

Nuclear Magnetic Resonance Studies on Unusual Bacterial Copolyesters of 3-Hydroxybutyrate and 4-Hydroxybutyrate

Yoshiharu Doi,* Masao Kunioka, Yoshiyuki Nakamura, and Kazuo Soga

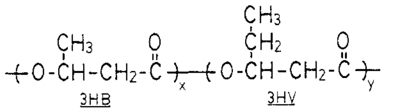
Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan. *Received November 4, 1987*

ABSTRACT: Unusual copolyesters of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) with a range of compositions varying from 0 to 49 mol % 4HB were produced by *Alcaligenes eutrophus* in a nitrogen-free culture solution of 4-hydroxybutyric or 4-chlorobutyric acid. The copolyesters were characterized by solution NMR, solid-state ^{13}C NMR, and differential scanning calorimetry. The compositions of the copolyesters were determined by 500-MHz ^1H NMR and two-dimensional homonuclear (^1H) correlated spectroscopy. The copolymers were shown to have a random sequence distribution of 3HB and 4HB units by analysis of the 125-MHz ^{13}C NMR spectra. The solid-state ^{13}C NMR spectra and thermal analyses showed that the crystallinity of copolyester decreases with an increase in the fraction of 4HB units.

Introduction

A wide variety of bacteria accumulate an optically active polyester, poly(3-hydroxybutyrate) (P(3HB)) as a carbon and energy source.[1-5] *Alcaligenes eutrophus* used in this study has a capability of accumulating P(3HB) in the cells in amounts up to 70% of the dry weight in nitrogen-limited cultures.[1,6] This P(3HB) is a thermoplastic with biodegradable, biocompatible, and optically active properties. Industrial-scale production of P(3HB) has begun.[7,8] The P(3HB) produced is exceptionally pure as a result of the biosynthetic process and contains no catalyst residues. Since this highly pure polyester makes an ideal material for the study of polymer crystallization, the thermodynamic properties of P(3HB) in crystallization have been investigated in detail.[9-11] The biosynthetic pathway of P(3HB) has been studied by enzymological methods, and several enzymes related to P(3HB) biosynthesis have been isolated from different bacteria.[12-16]

Recently, Imperial Chemical Industries (ICI) has developed a controlled fermentation for the production of an optically active copolyester of 3-hydroxybutyrate and 3-hydroxyvalerate (P(3HB-*co*-3HV))



$$\underbrace{-\!\!\left(O-\overset{CH_3}{CH}-CH_2-\overset{O}{\overset{\|}{C}}\right)_x}_{3HB}\underbrace{\left(O-\overset{CH_3}{\overset{|}{\overset{}{CH_2}}}\!\!\!\!\!\!\!\!\!\quad\;\; \overset{}{CH}-CH_2-\overset{O}{\overset{\|}{C}}\right)_y}_{3HV}$$

(1)

using propionic acid and glucose as the carbon sources for *A. eutrophus*.[17] The copolymer compositions were found to vary from 0 to 47 mol % 3HV units, depending on the composition of the carbon sources.[17,18] The biosynthetic pathway to P(3HB-*co*-3HV) in *A. eutrophus* has been investigated by using ^{13}C-labeled acetic and propionic acids as the carbon sources.[19,20] It has been suggested that the proportion of 3HV units is restricted to less than 50 mol % by a relatively fast metabolic pathway from propionyl to acetyl coenzyme A in the cells. Some of us have recently found that copolyesters with a wide range of compositions, up to 95 mol % 3HV units, are produced in *A. eutrophus* by using pentanoic and butyric acids as the carbon sources.[21,22]

These copolyesters have a statistically random distribution of 3HB and 3HV units, as shown by analysis of the ^{13}C NMR spectra.[23-25] The impact strength,[17] flexural modulus,[26] melting temperature,[23,25] and the rate of crystallization[18] of these copolyesters have been shown to be regulated by the content of 3HV units. Shear piezoelectricity was observed in oriented films of P(3HB-*co*-3HV)[27] and of P(3HB).[28] These bacterial polyesters may have application in various fields.

In this study we report the production of a new copolyester of 3-hydroxybutyrate and 4-hydroxybutyrate (P(3HB-*co*-4HB)) by *A. eutrophus* from 4-hydroxybutyric or 4-chlorobutyric acid. The molecular structure of P(3HB-*co*-4HB) in both solid and solution has been studied by analyses of the solid-state and solution NMR spectra.

Experimental Section

Biopolymer Synthesis. Poly(3-hydroxybutyrate) (P(3HB)) (sample 1) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) (samples 2–4) were isolated from *A. eutrophus* H16 (ATCC 17699) and *A. eutrophus* (NCIB 11599). The strains were maintained in 10% w/v glycerin at −20 °C. The bacteria were first grown at 30 °C in a nutrient-rich medium (1.0 dm^3) containing 10 g/dm^3 of yeast extract, 10 g/dm^3 of polypeptone, 5 g/dm^3 of meat extract, and 5 g/dm^3 of $(NH_4)_2SO_4$. The cells were harvested by centrifugation after 24 h and washed with water. Under these culture conditions the accumulation of polyesters in the cells was not observed. To promote polyester synthesis, about 3–4-g quantities of the washed cells were transferred into nitrogen-free mineral media[29] (1.0 dm^3, pH 7.0) containing different carbon sources (see Table I). The cells were cultivated in the media for 48 h at 30 °C, harvested by centrifugation, washed with acetone, and finally dried under vacuum at room temperature. Polyesters were extracted from dried cells with hot chloroform in a Soxhlet apparatus and purified by reprecipitation with hexane.

Analytical Procedures. The solution ^1H and ^{13}C NMR spectra of polyesters in chloroform were recorded on a JEOL GX-500 spectrometer. The 500-MHz ^1H NMR spectra were recorded at 27 °C on a $CDCl_3$ solution of polyester (3 mg/cm^3) with 3.5-μs pulse width ($\pi/4$ pulse angle), 5-s pulse repetition, 5000-Hz spectral width, 32K data points, and 500 accumulations. The 125-MHz ^{13}C NMR spectra were recorded at 27 °C on a $CDCl_3$ solution of polyester (25 mg/cm^3) with 10-μs pulse width ($\pi/4$ pulse angle), 5-s pulse repetition, 25000-Hz spectral width, 64K data points, and 10000–20000 accumulations. Tetramethylsilane (Me_4Si, $\delta = 0$) was used as an internal chemical shift standard.

The two-dimensional homonuclear (^1H) correlated (COSY) spectrum was recorded at 27 °C on a JEOL GX-500 spectrometer by using a $CDCl_3$ solution of polyester (3 mg/cm^3). A $\pi/2$–t_1–$\pi/2$ acquisition pulse sequence ($\pi/2 = 7.7\ \mu s$) was employed with appropriate phase cycling for quadrature detection with 1.25-s pulse repetition, 2500-Hz spectral width, and 1024 × 512 data matrix.

The solid-state CP/DD/MAS ^{13}C NMR spectra of polyesters in powder form were recorded at 67.8 MHz on a JEOL GX-270 spectrometer equipped with a CP-DD-MAS accessory at 27 °C with a 12-G decoupling field and 3.4-kHz spinning rate. Free induction decays were generated by using cross-polarization with a contact time of 10 ms and a recycle time of 2 s. The ^{13}C chemical shifts were referred to external adamantane (29.5 ppm from Me_4Si).

The melting temperatures (T_m) of polyester samples were recorded on a Seiko DSC-10. The samples of 3 mg were encapsu-

Table I
Production of Copolyesters of 3-Hydroxybutyrate (3HB) and 4-Hydroxybutyrate (4HB) by *Alcaligenes eutrophus* at 30 °C for 48 h

sample	strain	carbon source[a] (wt, g)	cell dry wt., g	polyester content,[b] wt %	copolyester[c] composition, mol %	
					3HB	4HB
1	ATCC 17699	$CH_3CH_2CH_2COOH$ (20)	9.6	51	100	0
2	ATCC 17699	$ClCH_2CH_2CH_2COOH$ (18)	5.1	27	89	11
3	ATCC 17699	$HOCH_2CH_2CH_2COOH$ (16.5)	3.7	30	67	33
4	NCIB 11599	$HOCH_2CH_2CH_2COOH$ (16.5)	4.5	20	51	49

[a] Carbon source in nitrogen-free culture media. (1.0 dm^3, pH 7.0). [b] Polyester content in dry cells. [c] Determined by ^{1}H NMR spectra.

Table II
Analytical Data of P(3HB-*co*-4HB) Samples

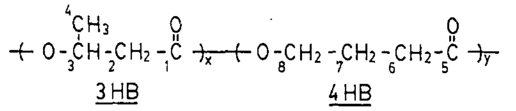
sample	$[\eta]$,[a] dL/g	T_m,[b] °C	ΔH_m,[b] cal/g	elementary analysis,[c] wt %		
				C	H	Cl
1	3.3	177	19.5	55.88	7.34	
2	3.9	160	11.1	55.50	7.09	0.29
3	2.9	166	7.1	55.60	6.64	
4	6.1	164	0.3	55.18	6.95	

[a] The intrinsic viscosity $[\eta]$ of copolyester was measured in chloroform at 30 °C. [b] T_m and ΔH_m were measured at 10 °C/min. [c] Calcd for $C_4H_6O_2$: C, 55.81; H, 7.02.

lated in aluminum pans and heated at 10 °C/min up to 200 °C. The heat of fusion of indium (6.80 cal/g) was used as a calorimetric calibration.

Results and Discussion

Production and Properties of P(3HB-*co*-4HB). The copolyesters (2) of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) were produced in *A. eutrophus* (ATCC 17699 and NCIB 11599) by using 4-hydroxybutyric acid ($HOCH_2CH_2CH_2COOH$) or 4-chlorobutyric acid ($ClCH_2CH_2CH_2COOH$) as the carbon source.



$$\underbrace{-\!\left(\mathrm{O}-\underset{3}{\overset{\overset{4}{\mathrm{CH_3}}}{\overset{|}{\mathrm{CH}}}}-\underset{2}{\mathrm{CH_2}}-\underset{1}{\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}}\right)_{x}}_{\text{3HB}}\underbrace{-\!\left(\mathrm{O}-\underset{8}{\mathrm{CH_2}}-\underset{7}{\mathrm{CH_2}}-\underset{6}{\mathrm{CH_2}}-\underset{5}{\overset{\mathrm{O}}{\overset{\|}{\mathrm{C}}}}\right)_{y}}_{\text{4HB}}$$

(2)

In this paper, the carbon species in the 3HB units are referred to as CO(1), CH_2(2), CH(3), and CH_3(4), respectively. The carbon species in the 4HB units are denoted by CO(5), CH_2(6), CH_2(7), and CH_2(8). The mole fractions of 3HB and 4HB units in the copolyesters were determined from the ^{1}H NMR spectra, as described in the following section.

Table I shows the fermentation conditions and compositions of three P(3HB-*co*-4HB) samples, together with the result of P(3HB) homopolymer biosynthesis from butyric acid. The contents of copolyesters in dry cells were in the range 20–30 wt %. The compositions of copolyester samples varied from 0 to 49 mol % 4HB, depending on the kinds of strains and carbon sources.

Table II gives the properties and elementary analysis data of these copolyester samples. The intrinsic viscosities of P(3HB-*co*-4HB) samples were in the range 2.9–6.1, indicative of the formation of copolyesters with a high degree of polymerization. The melting temperatures (T_m) did not decrease markedly from the value (179 °C) of P(3HB) with increasing fraction of 4HB units. In contrast, the enthalpies of fusion (ΔH_m) decreased with an increase in 4HB fraction, and the value of ΔH_m was almost zero at 49 mol % 4HB, indicating that the crystallinity of the copolyester decreases with the fraction of 4HB units. The elementary analysis data of three copolyester samples supported the composition of $C_4H_6O_2$. It is noted that the copolyester sample 2 from 4-chlorobutyric acid contains a negligible amount of chlorine. These elementary analysis data suggest that the same type of copolyester, P(3HB-*co*-4HB), was produced in *A. eutrophus* from different carbon sources of 4-hydroxybutyric and 4-chlorobutyric acids.

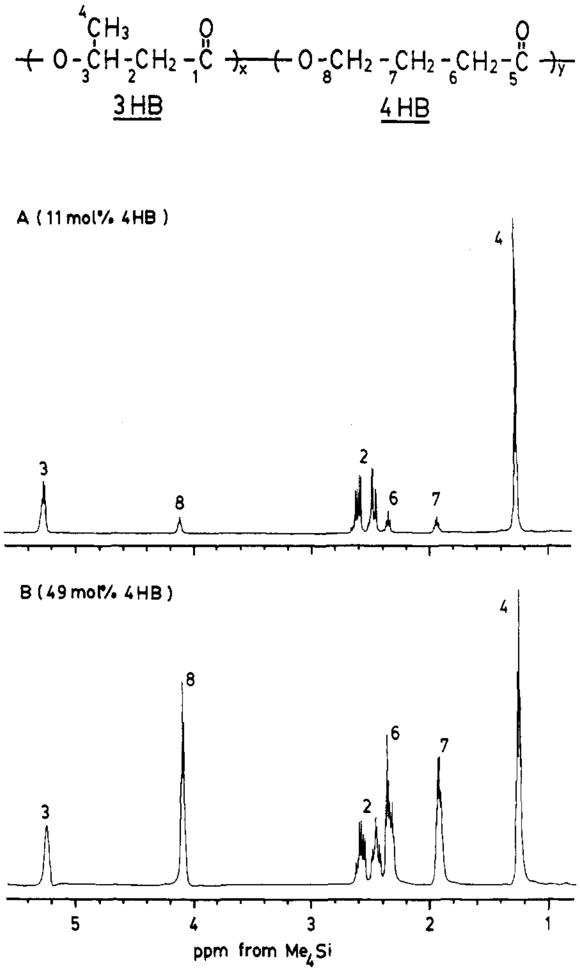


Figure 1. 500-MHz ^{1}H NMR spectra of copolyesters (samples 2 and 4) containing 3-hydroxybutyrate and 4-hydroxybutyrate units at 27 °C in chloroform. Chemical shifts are in ppm from Me_4Si.

^{1}H NMR Analysis. Figure 1 shows the 500-MHz ^{1}H NMR spectra of copolyester samples 2 (11 mol % 4HB) and 4 (49 mol % 4HB) in chloroform, together with the chemical shift assignment for each proton resonance. In addition to the well-characterized proton resonances (CH_3(4), CH_2(2), and CH(3)) in 3HB units,[30] three proton

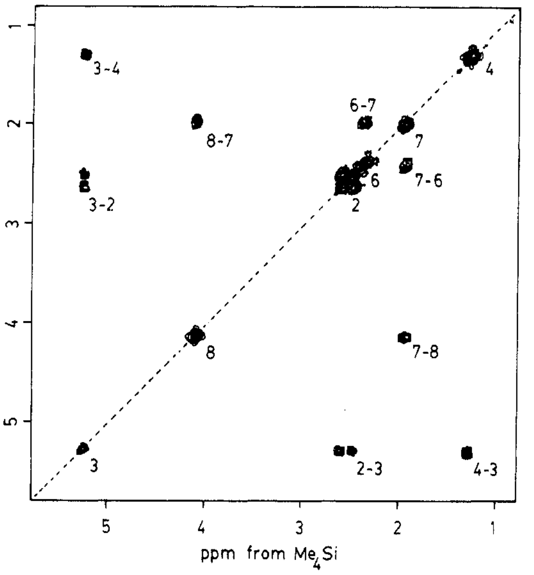


Figure 2. Two-dimensional homonuclear (^{1}H) correlated (COSY) spectrum of copolyester sample 4 in chloroform at 27 °C; *i*-*j* represents a spin group of *i* and *j* carbon species. Chemical shifts are in ppm downfield from Me$_4$Si.

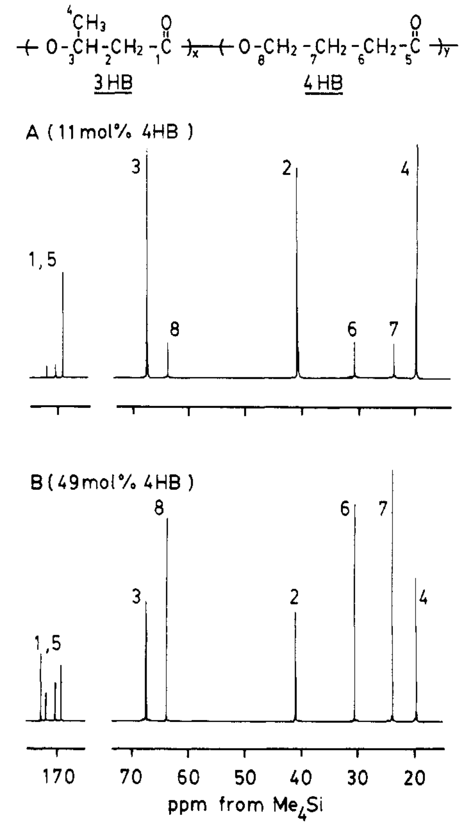


Figure 3. Proton-noise-decoupled 125-MHz ^{13}C NMR spectra of samples 2 and 4 in chloroform at 27 °C. Chemical shifts are in ppm from Me$_4$Si.

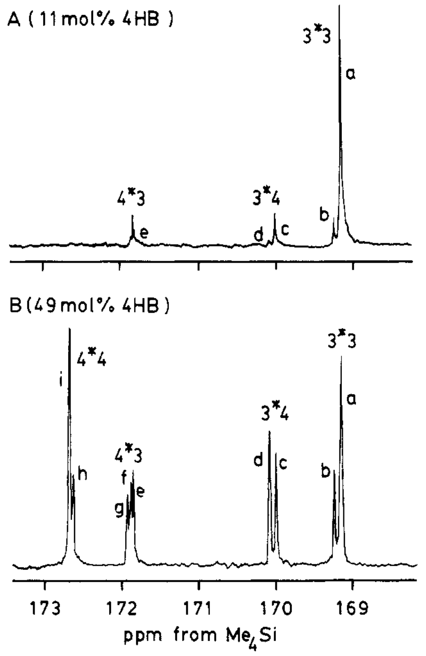


Figure 4. Expanded ^{13}C NMR spectra at 125 MHz for carbonyl resonances of samples 2 and 4. The ^{13}C chemical shift assignments are given in Table III.

resonances (7, 6, and 8) appear with identical intensities. In the spectrum A of sample 2 (11 mol % 4HB) from 4-chlorobutyric acid, a pentuplet proton resonance of CH$_2$(7) at 1.97 ppm and two triplet proton resonances of CH$_2$(6) at 2.39 ppm and CH$_2$(8) at 4.12 ppm can be detected. These three resonances were assigned to the methylene proton resonances in 4HB units by a comparison of ^{1}H NMR spectrum of 4-hydroxybutyric acid in D$_2$O. The spectrum B of sample 4 (49 mol % 4HB) from 4-hydroxybutyric acid shows six proton resonances at identical chemical shifts to those in spectrum A. However, the splitting patterns of the proton resonances in spectrum B are more complicated than those in spectrum A. For example, the CH$_3$(4) proton resonance of sample 2 consists of a doublet as expected, but the CH$_3$(4) proton resonance of sample 4 appears to consist of a quartet. The other proton resonances of sample 4 are also resolved into more complicated patterns than those of sample 2. To examine the composition of sample 4, the two-dimensional homonuclear (^{1}H) correlated (COSY) NMR was obtained.

Figure 2 shows the 500-MHz 2D ^{1}H-COSY NMR spectrum of sample 4 (49 mol % 4HB). In the region of three methylene proton resonances in 4HB units, two spin groups of 6–7 and 7–8 were detected and other spin relations were not observed, indicating that the order of carbon sequence in 4HB units is 6–7–8. On the other hand, two spin groups of 2–3 and 3–4 were identified in relation of 3HB units, confirming that the order of carbon sequence in 3HB units is 2–3–4. Thus, the 2D ^{1}H-COSY NMR spectrum demonstrated that the composition of sample 4 as well as sample 2 is represented by the structure (**2**). The complicated patterns of proton resonances in the spectrum B of sample 4 may be due to a difference in the chemical shift of each proton resonance in different dyad sequences of connecting 3HB and 4HB units.

The mole fractions of two monomeric units in copolyester samples were determined from the intensity ratio of proton resonances in 3HB and 4HB units. The result is given in Table I.

Solution ^{13}C NMR Analysis. Figure 3 shows the 125-MHz ^{13}C NMR spectra of samples 2 and 4, together with the chemical shift assignment for each carbon resonance. The six ^{13}C resonances at 15–70 ppm could be assigned to specific carbon species in 3HB and 4HB units, as shown in Figure 3. The proton off-resonance decoupled ^{13}C NMR spectrum of sample 2 supported the chemical shift assignments of the six ^{13}C resonances.

The expanded spectra of carbonyl carbon resonances at 169–173 ppm are shown in Figure 4. The carbonyl resonances (1 and 5) of sample 2 (11 mol % 4HB) are resolved into three groups of peaks, and those of sample 4 (49 mol % 4HB) are clearly resolved into four groups of peaks,

Table III
Chemical Shifts and Relative Intensities of ^{13}C Resonances in P(3HB-*co*-4HB) Samples

carbon species	peak[a]	chemical shift, ppm	sequence	relative intensities: sample 2 obsd	relative intensities: sample 2 calcd[b]	relative intensities: sample 4 obsd	relative intensities: sample 4 calcd[b]
$CH_3(4)$	a	19.77	33*3	0.79	0.79	0.43	0.35
	b	19.82	33*4	0.09	0.10	0.18	0.24
	c	19.87	43*3	0.09	0.10	0.17	0.24
	d	19.93	43*4	0.03	0.01	0.22	0.17
$CH_2(7)$	a	23.95	34*	0.90	0.89	0.43	0.41
	b	24.00	44*	0.10	0.11	0.57	0.59
$CH_2(6)$	a	30.59	34*4	0.09	0.10	0.17	0.24
	b	30.66	44*4	0.04	0.01	0.46	0.34
	c	30.79	34*33	0.71	0.70	0.16	0.11
	d	30.82	34*34	0.08	0.09	0.08	0.07
	e	30.85	44*33	0.07	0.09	0.04	0.14
	f	30.88	44*34	0.01	0.01	0.09	0.10
$CH_2(2)$	a	40.54	33*4	0.08	0.10	0.21	0.24
	b	40.65	43*4	0.02	0.01	0.22	0.17
	c	40.80	33*33	0.71	0.70	0.32	0.21
	d	40.86	33*34	0.10	0.09	0.10	0.14
	e	40.90	43*33	0.08	0.09	0.09	0.14
	f	40.95	43*34	0.01	0.01	0.06	0.10
$CH_2(8)$	a	63.54	44*	0.12	0.11	0.60	0.59
	b	63.64	34*	0.88	0.89	0.40	0.41
CH(3)	a	67.42	43*	0.10	0.11	0.38	0.41
	b	67.62	33*	0.90	0.89	0.62	0.59
CO(1)	a	169.15	33*3	0.75	0.70	0.22	0.18
	b	169.25	43*3	0.07	0.09	0.10	0.12
	c	170.00	33*4	0.08	0.09	0.10	0.12
	d	170.08	43*4	0.01	0.01	0.11	0.09
CO(5)	e	171.85	4*3	0.09	0.10	0.19	0.21
	f	171.88	4*3				
	g	171.92	4*3				
	h	172.62	34*4	0	0.01	0.09	0.12
	i	172.67	44*4	0	0	0.19	0.16

[a] Peaks in Figures 4 and 5. [b] Calculated with the values of p_{ij} in Table IV.

arising from different dyad sequences of 3HB and 4HB units. The peaks a and b at 169.15–169.25 ppm are assignable to the carbonyl resonances in the 3*3 (3HB*–3HB) sequence, since the chemical shifts are almost consistent with that (169.16 ppm) of the carbonyl resonance in P(3HB) homopolymer. The peaks h and i at the lowest magnetic field (172.62–172.67 ppm) are assignable to the carbonyl resonances in the 4*4 (4HB*–4HB) sequence, since these peaks are not observed in the spectrum A of sample 2 with a relatively low 4HB content (11 mol %). The peaks c and d at 170.00–170.08 ppm and the peaks e, f, and g at 171.85–171.92 ppm are assignable to the carbonyl resonances in the 3*4 (3HB*–4HB) and 4*3 (4HB*–3HB) sequences, respectively.

The peaks from a to i except for e, f, and g can be assigned to the carbonyl resonances in different triad sequences of 3HB and 4HB units. For example, peaks a and b are assignable to the carbonyl resonance in 33*3 and 43*3 sequences, respectively. The peaks e, f, and g may be assigned to the carbonyl resonances in different tetrad sequences (34*33, 34*34, 44*33, and 44*34). The ^{13}C chemical shift assignments of peaks a–i in the carbonyl resonances are given in Table III, together with the relative area of each peak.

The other six carbon resonances are split into several peaks as well as the carbonyl resonances. Figure 5 shows the expanded 125-MHz ^{13}C NMR spectrum for six carbon resonances of sample 4 (49 mol % 4HB). The chemical shift assignment and relative area of each peak in the respective ^{13}C resonance are given in Table III. The chemical shift assignments have been made by a comparison of the relative areas of all peaks in the ^{13}C NMR spectra of copolyester samples 2 and 4 with different compositions. The resonances of $CH_2(7)$, $CH_2(8)$, and CH(3) carbons are resolved into two peaks due to the different dyad sequences of 3HB and 4HB units. The resonance of the $CH_3(4)$ carbon is resolved into four peaks, while the resonances of $CH_2(6)$ and $CH_2(2)$ carbons are

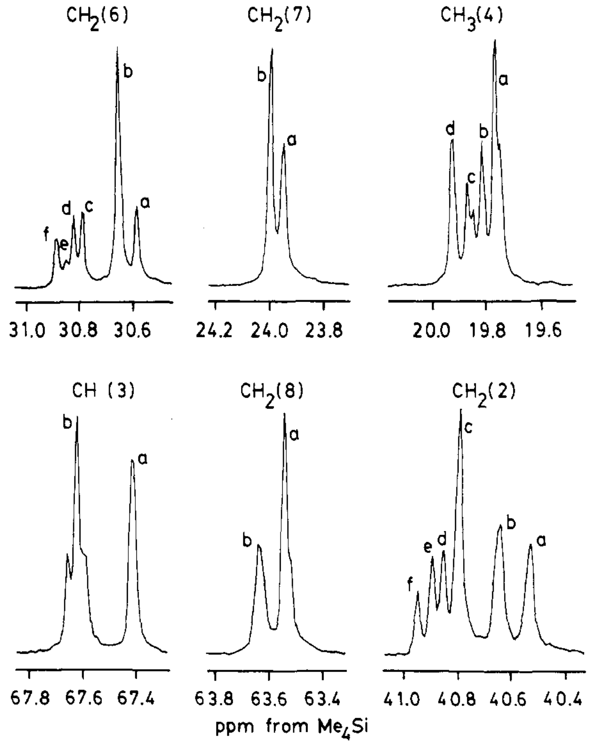


Figure 5. Expanded ^{13}C NMR spectrum at 125 MHz of sample 4. The ^{13}C chemical shift assignments are given in Table III.

Table IV
Four Conditional Probabilities p_{ij} and Reactivity Ratio Product r_3r_4

sample	p_{33}	p_{34}	p_{43}	p_{44}	r_3r_4[a]
2	0.89	0.11	0.89	0.11	1.0
4	0.59	0.41	0.42	0.58	2.0

[a] $r_3r_4 = p_{33}p_{44}/p_{34}p_{43}$.

resolved into six peaks due to triad and tetrad sequences of 3HB and 4HB units.

The observed dyad, triad, and tetrad sequence distributions of 3HB and 4HB units may be interpreted in terms of the statistics of a binary copolymerization involving the following propagation steps:

$$3^* + 3\text{-CoA} \xrightarrow{k_{33}} 33^* + \text{CoA} \quad (1)$$

$$3^* + 4\text{-CoA} \xrightarrow{k_{34}} 34^* + \text{CoA} \quad (2)$$

$$4^* + 3\text{-CoA} \xrightarrow{k_{43}} 43^* + \text{CoA} \quad (3)$$

$$4^* + 4\text{-CoA} \xrightarrow{k_{44}} 44^* + \text{CoA} \quad (4)$$

In the above scheme, CoA is coenzyme A, k_{ij} is the rate constant of step ij, and the numerals 3 and 4 refer to 3HB and 4HB units, respectively. Since the monomer concentrations of 3-CoA and 4-CoA in a cell are not known, we use here the conditional probability p_{ij} of step ij with the relation that $p_{33} + p_{34} = 1$ and $p_{43} + p_{44} = 1$, as represented by

$$p_{33} = k_{33}[\text{3-CoA}]/(k_{33}[\text{3-CoA}] + k_{34}[\text{4-CoA}]) \quad (5)$$

$$p_{44} = k_{44}[\text{4-CoA}]/(k_{43}[\text{3-CoA}] + k_{44}[\text{4-CoA}]) \quad (6)$$

Assuming a first-order Markovian process for the above copolymerization, one can express different dyad, triad, and tetrad fractions of 3HB and 4HB units with four conditional probabilities.[31]

$$F_{33} = p_{43}p_{33}/(p_{34} + p_{43}) \quad (7)$$

$$F_{34} = F_{43} = p_{43}p_{34}/(p_{34} + p_{43}) \quad (8)$$

$$F_{44} = p_{34}p_{44}/(p_{34} + p_{43}) \quad (9)$$

$$F_{333} = p_{33}F_{33}; \quad F_{334} = F_{433} = p_{33}F_{34};$$
$$F_{343} = p_{43}F_{34}; \quad F_{434} = p_{34}F_{34};$$
$$F_{443} = F_{344} = p_{43}F_{44}; \quad F_{444} = p_{44}F_{44} \quad (10)$$

$$F_{3333} = p_{33}F_{333}; \quad F_{3334} = F_{4333} = p_{34}F_{333};$$
$$F_{3433} = p_{43}F_{433}; \quad F_{4334} = p_{34}F_{433};$$
$$F_{4433} = p_{44}F_{433}; \quad F_{3434} = p_{34}F_{343};$$
$$F_{4434} = p_{44}F_{434} \quad (11)$$

Combining the above relations with the observed sequence distributions, we determined the four conditional probabilities p_{ij}. The values of p_{ij} are listed in Table IV, together with the reactivity ratio product, r_3r_4. For comparison, the dyad, triad, and tetrad distributions calculated with the values of p_{ij} are given in Table III. The calculated sequence distributions are in good agreement with the observed distributions for both samples 2 and 4, which supports the ^{13}C chemical shift assignment of each peak in Table III. The observed values of the reactivity ratio product r_3r_4 are in the range 1.0–2.0, which indicates that the sequence distribution of 3HB and 4HB units is close to a statistically random distribution.

Solid-State CP/MAS ^{13}C NMR Analysis. Figure 6 shows the CP/MAS ^{13}C NMR spectra at 67.8 MHz of three copolyester samples (11, 33, and 49 mol % 4HB) in powder form, observed at a CP contact time of 10 ms. Table V gives the chemical shifts of ^{13}C resonances for three copolyester samples, together with those of P(3HB) homopolymer sample 1.

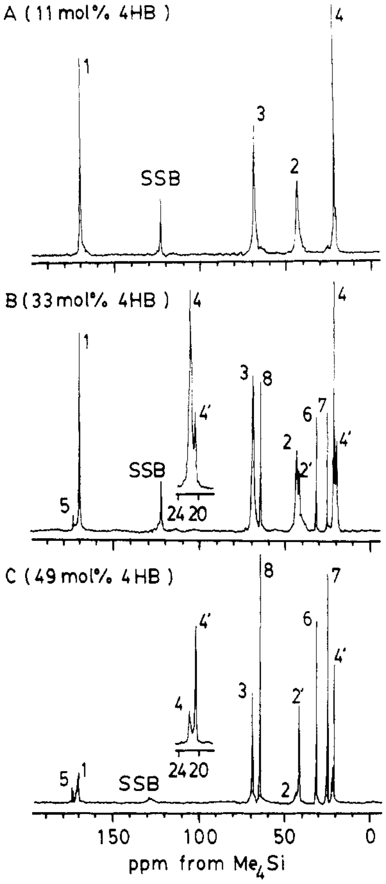


Figure 6. CP/MAS ^{13}C NMR spectra at 67.8 MHz of copolyesters (samples 2, 3, and 4) in powder form. Chemical shifts are in ppm downfield from Me$_4$Si. Here, SSB denotes the spinning side band of CO resonance. The ^{13}C chemical shift assignments are given in Table V.

Table V
CP/MAS ^{13}C NMR Chemical Shifts for Polyester Samples in Powder Form

	^{13}C chemical shift from Me$_4$Si, ppm			
species	sample 1	sample 2	sample 3	sample 4
$CH_3(4)$	21.3	21.6	21.5	21.5
$CH_3(4')$		20.5	20.3	20.2
$CH_2(2)$	42.8	42.9	43.0	43.1
$CH_2(2')$			41.3	41.4
CH(3)	68.4	68.7	68.6	68.3
CO(1)	169.8	170.0	169.9	170.0
$CH_2(7)$			24.9	24.9
$CH_2(6)$			31.2	31.2
$CH_2(8)$			64.2	64.1
CO(5)			173.2	172.6

In the spectrum A of sample 2 (11 mol % 4HB) the resonances of carbon species (1–4) in 3HB units appear at almost identical chemical shifts with those of ^{13}C resonances in P(3HB) homopolymer (see Table V), and the resonances of carbon species (5–8) in 4HB units are hardly detectable. In spectra B and C of samples 3 (33 mol % 4HB) and 4 (49 mol % 4HB), the resonances 7, 6, 8, and 5 of carbon species in 4HB units appear at 24.9, 31.2, 64.1, and 172.6 ppm, respectively. The peaks of ^{13}C resonances in 4HB units are very sharp, and their chemical shifts are approximately consistent with those of ^{13}C resonances in the solution ^{13}C NMR spectra of copolyester samples in Table III. The methyl carbon resonance of 3HB units splits into two peaks, 4 and 4′. Peak 4 at 21.5 ppm is consistent with the $CH_3(4)$ carbon resonance (21.3 ppm) in the solid-state spectrum of P(3HB) powder, while the chemical shift (20.2 ppm) of peak 4′ is approximately consistent with that (19.8 ppm) of the $CH_3(4)$ carbon resonance in the solution spectrum of P(3HB) in chloroform. The relative intensity of peaks 4 to 4′ decreases with an increase in the fraction of 4HB units. In addition, the methylene carbon resonance of 3HB units splits into peaks 2 (43.1 ppm) and 2′ (41.4 ppm). The chemical shift of peak 2′ is almost identical with the value (40.8 ppm) of $CH_2(2)$ carbon resonance in the solution spectrum of P(3HB) in chloroform. The relative intensity of peaks 2 to 2′ decreases with increasing 4HB fraction as well as the intensity of peaks 4 to 4′.

In the CP/MAS ^{13}C NMR spectrum C of sample 4 (49 mol % 4HB) the intensities of carbonyl carbon resonances (1 and 5) are very weak in comparison with those of other carbon resonances, indicating that the cross-polarization arising from ^{13}C–^{1}H dipole interaction is ineffective because of a high mobility of copolyester segments.

The P(3HB) polymer chain crystallizes as a left-handed 2_1 helix in the solid state.[32,33] The results of CP/MAS ^{13}C NMR analysis indicate that the crystallinity of the P(3HB-*co*-4HB) sample decreases with an increasing fraction of 4HB units. Peaks 4 and 2 in the CP/MAS ^{13}C NMR spectra of the P(3HB-*co*-4HB) samples arise from the $CH_3(4)$ and $CH_2(2)$ carbon species of 3HB units in the crystalline phase with a regular conformation of 2_1 helix. On the other hand, peaks 4′ and 2′ are likely to arise from those of 3HB units, which are poorly ordered with a high flexibility in the amorphous phase. The decrease of crystallinity in the P(3HB-*co*-4HB) samples was also suggested by the enthalpy of fusion, ΔH_m in table II. It is noted that the property of P(3HB-*co*-4HB) in the solid state is different from the solid-state property of P(3HB-*co*-3HV). Marchessault et al.[23] have shown that the P(3HB-*co*-3HV) samples have high crystallinities (>60%) throughout a range of compositions of 0–47 mol % 3HV.

In conclusion, new bacterial copolyesters are described that have a random sequence distribution of 3HB and 4HB units, and the crystallinity decreases with an increasing fraction of 4HB units.

Registry No. P(4HB) (SRU), 28728-97-4; P(4HB) (homopolymer), 114959-05-6; poly(4-chlorobutyric acid) (homopolymer), 114959-06-7.

References and Notes

(1) Schlegel, H. G.; Gottschalk, G.; Bartha, R. V. *Nature (London)* **1961**, *191*, 463.
(2) Lundgren, D. G.; Alper, R.; Schnaitman, C.; Marchessault, R. H. *J. Bacteriol.* **1965**, *89*, 245.
(3) Griebel, R.; Smith, Z.; Merrick, J. M. *Biochemistry* **1968**, *7*, 3676.
(4) Saito, T.; Fukui, T.; Ikeda, F.; Tanaka, Y.; Tomita, K. *Arch. Microbiol.* **1977**, *114*, 211.
(5) Suzuki, T.; Yamane, T.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1986**, *24*, 370.
(6) Henzle, E.; Lafferty, R. M. *Eur. J. Appl. Microbiol. Biotechnol.* **1980**, *11*, 8.
(7) Howells, E. R. *Chem. Ind. (London)* **1982**, *7*, 508.
(8) King, P. P. *J. Chem. Tech. Biotech.* **1982**, *32*, 2.
(9) Marchessault, R. H.; Coulombe, S.; Morikawa, H.; Okamura, K.; Revol, J. R. *Can. J. Chem.* **1981**, *59*, 38.
(10) Barham, P. J.; Keller, A.; Otum, E. L.; Holmes, P. A. *J. Mater. Sci.* **1984**, *19*, 2781.
(11) Barham, P. J. *J. Mater. Sci.* **1984**, *19*, 3826.
(12) Merrick, J. M.; Doudoroff, M. *Nature (London)* **1961**, *189*, 890.
(13) Senior, P. J.; Dowes, E. A. *Biochem. J.* **1973**, *134*, 225.
(14) Oeding, V.; Schlegel, H. G. *Biochem. J.* **1973**, *134*, 239.
(15) Fukui, T.; Yoshimoto, A.; Matsumoto, M.; Hosokawa, S.; Saito, T.; Nishikawa, H.; Tomita, K. *Arch. Microbiol.* **1976**, *110*, 149.
(16) Nishimura, T.; Saito, T.; Tomita, K. *Arch. Microbiol.* **1978**, *116*, 21.
(17) Holmes, P. A. *Phys. Technol.* **1985**, *16*, 32.
(18) Bloembergen, S.; Hamer, G. K.; Bluhm, T. L.; Marchessault, R. H. *Macromolecules* **1985**, *19*, 2865.
(19) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *J. Chem. Soc., Chem. Commun.* **1986**, 1696.
(20) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1987**, *20*, 2988.
(21) Doi, Y.; Tamaki, A.; Kunioka, M.; Soga, K. *J. Chem. Soc., Chem. Commun.* **1987**, 1635.
(22) Doi, Y.; Tamaki, A.; Kunioka, M.; Soga, K. *Appl. Microbiol. Biotechnol.* *1988*, *28*, 330.
(23) Bluhm, T. L.; Hamer, G. K.; Marchessault, R. H.; Fyfe, C. A.; Veregin, R. P. *Macromolecules* **1986**, *19*, 2871.
(24) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1986**, *19*, 2860.
(25) Doi, Y.; Kunioka, M.; Tamaki, A.; Nakamura, Y.; Soga, K. *Makromol. Chem.*, in press.
(26) Owen, A. J. *Colloid Polym. Sci.* **1985**, *263*, 799.
(27) Fukuda, E.; Ando, Y. *Int. J. Biol. Macromol.* **1986**, *8*, 361.
(28) Ando, Y.; Fukuda, E. *J. Polym. Sci., Polym. Phys. Ed.* **1984**, *22*, 1821.
(29) Repaske, R.; Repaske, A. C. *Appl. Environ. Microbiol.* **1976**, *32*, 585.
(30) Doi, Y.; Kunioka, M.; Nakamura, Y.; Soga, K. *Macromolecules* **1986**, *19*, 1274.
(31) Randall, J. C. *Polymer Sequence Determination*; Academic: New York, 1977; Chapter 4.
(32) Cornibert, J.; Marchessault, R. H. *J. Mol. Biol.* **1972**, *71*, 735.
(33) Yokouchi, M.; Chatani, Y.; Tadokoro, H.; Teranishi, K.; Tani, H. *Polymer* **1973**, *23*, 694.

Laser Flash Photolysis of a 4,4′-Methylenebis(phenyl isocyanate) (MDI) Based Polyurethane and Model Carbamates

C. E. Hoyle,*,[†] Y. G. No,[†] K. G. Malone,[†] S. F. Thames,[†] and David Creed[‡]

Department of Polymer Science and Department of Chemistry, University of Southern Mississippi, Hattiesburg, Mississippi 39406. *Received December 7, 1987*

ABSTRACT: Transient species in the laser flash photolysis of solutions of 4,4′-methylenebis(phenyl isocyanate) (MDI) based polyurethanes and small molecule models have been detected. One of the transient species, a substituted anilinyl radical, is not quenched by oxygen, while a second transient is extinguished by the introduction of oxygen. Based on the spectral results, a plausible mechanism is offered for the primary photochemical reactions of MDI-based polyurethanes.

The photolytic degradation of polyurethanes based on aromatic diisocyanates continues to receive a considerable amount of attention.[1-7] Indeed, there have been a number of schemes proposed to account for their photolytic degradation, each having its own merit.[1-7] This paper is designed to provide basic insight into the nature of the transient intermediates produced during the primary

---

[†] Department of Polymer Science.
[‡] Department of Chemistry.

0024-9297/88/2221-2727$01.50/0