Biodegradation of Microbial Copolyesters: Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) and Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)

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ABSTRACT: Hydrolytic and enzymatic degradation processes of microbial copolyesters, poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), were studied by monitoring the time-dependent changes in molecular weights and weight loss (erosion) of copolyester films. Hydrolysis studies on a series of microbial copolyesters were carried out on the solution-cast films in 0.01 M phosphate buffer at 55 °C and pH 7.4. The weights of all copolyester films were unchanged over 58 days, while the molecular weights decreased with time. A random chain scission by hydrolysis took place throughout the whole polymer matrix. The rates of random chain scission in P(3HB-*co*-4HB) films were faster than those in P(3HB-*co*-3HV) films. The enzymatic degradations of copolyester films were studied at 37 °C and pH 7.5 in the aqueous solution of the extracellular P(3HB) depolymerase purified from *Alcaligenes faecalis* T1. The rate of enzymatic degradation on the respective copolyester films was much faster than the rate of simple hydrolytic degradation. The enzymatic degradation occurred at the surface of the copolyester film. The surface erosion by P(3HB) depolymerase was confirmed by the scanning electron micrographs of P(3HB) film. The rate of enzymatic surface erosion decreased in the order P(3HB-*co*-4HB) > P(3HB) > P(3HB-*co*-3HV). It has been found that the presence of 4HB units in polyesters accelerates the rates of both hydrolytic and enzymatic degradations.

Introduction

A variety of bacteria produce poly(3-hydroxybutyrate), P(3HB), as an intracellular storage polymer of carbon and energy under various nutritional and environmental conditions.[1] The polyester is accumulated as distinct granules in the cells, and it can be isolated from cells by means of solvent extraction[2] and hypochlorite treatment.[3] The isolated P(3HB) is a biodegradable and biocompatible thermoplastic.[4] Some bacteria excrete extracellular P(3HB) depolymerases to degrade environmental P(3HB) and utilize the decomposed compounds as nutrients.[5,6] The extracellular P(3HB) depolymerases have been isolated from *Pseudomonas lemoignei*[7,8] and *Alcaligenes faecalis*.[9-11] Recently, the extracellular P(3HB) depolymerase gene from *A. faecalis* was cloned into *Escherichia coli* and its DNA sequence was determined.[12]

A microbial copolyester of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV) has been produced by *Alcaligenes eutrophus* from propionic acid[4] or pentanoic acid.[13] The copolyester has been shown to have a statistically random distribution of 3HB and 3HV units.[14,15,21] Recently, the microbial copolyester of 3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) has been produced by *A. eutrophus* from 4-hydroxybutyric acid,[16,17] 4-chlorobutyric acid,[16] 1,4-butanediol,[18] or γ-butyrolactone.[18] The mechanical and physical properties of these two families of copolyesters have been shown to be regulated by the copolymer compositions.[19-24] The microbial copolyesters can be formed into films, fibers, and sheets. These products are expected to be degradable in the environment, either by hydrolytic or enzymatic degradation processes.

In this paper, first, we report the hydrolytic degradation of microbial copolyester films of P(3HB-*co*-3HV) and P(3HB-*co*-4HB) in aqueous buffer solution of pH 7.4. The degradation processes are studied by monitoring the time-dependent changes in molecular weights and weight loss (erosion) of copolyester films. Second, we report the enzymatic degradation of microbial polyester films by the extracellular P(3HB) depolymerase, which was isolated from *A. faecalis*. Finally, the degradation processes of microbial polyesters and the effects of polymer compositions on the degradation rates are discussed.

Experimental Section

Microbial Polyester Preparation. We prepared seven polyester samples of varying molecular weights and compositions. Samples 1 and 2 of P(3HB) homopolyester were isolated from *A. eutrophus*[14] and *Zoogloea ramigera*,[25] respectively. Samples 3 and 4 of P(3HB-*co*-3HV) copolyesters (3HV = 45 and 71 mol %) were produced in *A. eutrophus* from pentanoic and butyric acids.[13] Samples 5–7 of P(3HB-*co*-4HB) copolyesters (4HB = 10, 17, and 27 mol %) were produced in *A. eutrophus* from 4-hydroxybutyric and butyric acids.[17] The compositions of copolyesters were measured by ^{1}H NMR spectroscopy.[14,16] Table I shows the compositions and molecular weights of polyester samples used in this study. The films of polyester samples were prepared by conventional solvent-casting techniques from chloroform solutions of polyesters using glass Petri dishes as casting surfaces. The solution-cast films were aged for 3 weeks to reach equilibrium crystallinity prior to analysis.[20]

Hydrolytic Degradation. Degradation studies of polyester films were carried out at 55 °C in a 0.01 M phosphate buffer (pH 7.4). Polyester films (initial disk dimensions, 12-mm diameter and 0.04–0.07 mm thick) were placed in small bottles containing 40 cm^3 of buffer and maintained at 55 ± 0.1 °C. Samples were periodically removed, washed with distilled water, and dried to constant weight in vacuo before analysis.

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Enzymatic Degradation. The extracellular P(3HB) depolymerase was purified to electrophoretic homogeneity from *A. faecalis* T1 as described in a previous paper.[10] The enzymatic degradation of polyester films was carried out at 37 °C in a 0.1 M phosphate buffer (pH 7.5). Polyester films (initial weights, 5.0–8.0 mg; initial film dimensions, 10 × 10 mm in size and 0.04–0.07 mm thick) were placed in small bottles containing 1.0 cm^3 of buffer. The reaction was started by the addition of 5 μL of aqueous solution of P(3HB) depolymerase (3 μg). The reaction solution was incubated at 37 ± 0.1 °C with shaking. Samples were periodically removed, washed with ethanol, and dried to constant weight in vacuo.

Analytical Procedures. All molecular weight data were obtained at 40 °C by using a Shimadzu 6A GPC system and a 6A refractive index detector with a Shodex 80M column. Chloroform was used as eluant at a flow rate of 0.5 cm^3/min, and sample concentration of 1.0 mg/cm^3 was used. Polystyrene standards with a low polydispersity were used to make a calibration curve.

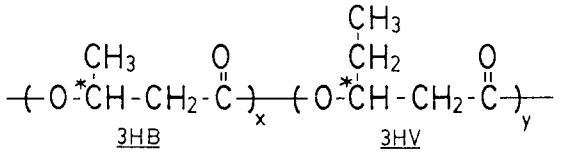
The ^1H NMR analyses of polyester samples were carried out on a JEOL FX-100 spectrometer. The 100-MHz ^1H NMR spectra were recorded at 27 °C in $CDCl_3$ solution of polyester (5 mg/cm^3) with 45° pulse (15 μs), 5-s pulse repetition, 1000-Hz spectral width, 8K data points, and 200 accumulations.

Wide-angle X-ray diffraction measurements of polyester samples were made on a Rigaku RAD-1VB system. Cu Kα radiation (λ = 0.1542 nm) was used as the source. The X-ray diffraction patterns of polyester films were recorded at 27 °C in the range of 2θ = 6–40° at a scan speed of 1.0°/min, and X-ray crystallinities were determined according to Vonk's method.[26]

Surfaces and cross-sectional appearances of polyester films were observed with a scanning electron microscope (JEOL JSM-T220) after Au coating of the films using an ion coater.

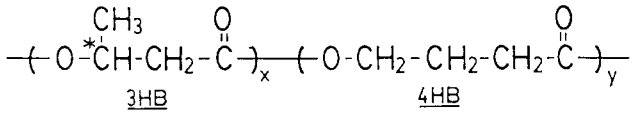
Results and Discussion

Table I summarizes compositions, molecular weights, and crystallinities of microbial polyester films used in this study. Samples 1 and 2 are P(3HB) homopolyesters with different molecular weights ($\overline{M}_n$ = 768 000 and 22 000). Samples 3 and 4 are copolyesters of 3-hydroxybutyrate and 3-hydroxyvalerate, P(3HB-*co*-3HV) (**1**). Samples 5–7 are copolyesters of 3-hydroxybutyrate and



(1)

4-hydroxybutyrate, P(3HB-co-4HB) (**2**). The X-ray crystallinities of polyester films varied from 40 to 59%, depending on the compositions of copolyesters.



(2)

Hydrolytic Degradation. Hydrolysis studies on a series of microbial copolyesters were carried out on solution-cast films at 55 °C in a 0.01 M phosphate buffer (pH 7.4). Table II lists the weight loss (erosion) data of five copolyester films as a function of degradation time, and Table III gives the molecular weight data.

The weights of all copolyester films were unchanged over a period of 58 days, indicating that no polymer erosion occurs at 55 °C. On the other hand, the molecular weights of copolyesters decreased with time. Figure 1 shows the ratio of number-average molecular weight

Table I
Compositions and Properties of Microbial Polyester Films

sample no.	compositn,[a] mol %			mol wt[b]		crystallinity,[c] %
	3HB	3HV	4HB	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$	
1	100	0	0	768	1.9	59 ± 5
2	100	0	0	22	3.4	55 ± 5
3	55	45	0	369	2.6	53 ± 5
4	29	71	0	254	2.0	56 ± 5
5	90	0	10	395	3.0	41 ± 5
6	83	0	17	366	2.9	43 ± 5
7	73	0	27	332	2.4	40 ± 5

[a] Determined by ^1H NMR. [b] Determined by GPC. [c] Determined by X-ray diffraction.

$\overline{M}_n$ at time t to initial $\overline{M}_n$[$\overline{M}_n(t)/\overline{M}_n(0)$] for the respective copolyester film during the hydrolytic degradation. The rate of molecular weight loss is apparently influenced by the copolyester compositions and decreases in the series

P(3HB-*co*-27% 4HB) > P(3HB-*co*-17% 4HB) > P(3HB-*co*-10% 4HB) > P(3HB-*co*-45% 3HV) > P(3HB-*co*-71% 3HV) (1)

The average number of bond cleavages per original polymer molecule, N, is given by eq 2

$$N = \overline{M}_n(0)/\overline{M}_n(t) - 1 \quad (2)$$

where $\overline{M}_n(0)$ and $\overline{M}_n(t)$ represent, respectively, the values of $\overline{M}_n$ at degradation time zero and time t. If the chain scission is completely random, the value of N is anticipated to be a linear function of time t as[27]

$$N = \overline{M}_n(0)/\overline{M}_n(t) - 1 = k_d\overline{P}_n(0)t \quad (3)$$

where k_d is the rate constant of hydrolytic degradation, and $\overline{P}_n(0)$ is the number-average degree of polymerization at time zero.

Figure 2 shows a linear relationship between the N value and time t for copolyester samples 3–7, which confirms that the decrease in $\overline{M}_n$ is due to random chain scission. Table IV lists the values of degradation rate constant k_d for copolyester samples. The largest value (1.7×10^{-5} day^{-1}) of k_d was observed for the P(3HB-*co*-27% 4HB) film, while the smallest value (2.3×10^{-6} day^{-1}) was observed for the P(3HB-*co*-71% 3HV) film. In all copolyester samples the molecular weight distributions of samples were unimodal and narrow during the chain scission by hydrolysis (see Table III).

Thus, our results indicate that a random chain scission by hydrolysis proceeds throughout the whole polymer matrix and that the chain scission rate is strongly dependent upon the compositions of copolyesters.

Enzymatic Degradation. The extracellular P(3HB) depolymerase was purified to electrophoretic homogeneity from *A. faecalis* T1[10] and used in this study. The enzymatic degradation of polyesters was carried out on solution-cast films (initial weights 5–8 mg) at 37 °C in the aqueous solution of P(3HB) depolymerase (3 μg) over a period of 20 h. It has been confirmed that no polyester erosion occurs at 37 °C in the absence of P(3HB) depolymerase.

Figure 3 shows the weight loss (erosion) profiles of polyester films as a function of degradation time. The weight of film erosion, W, increases proportionally to time t as

$$W = k_e t \quad (4)$$

given in eq 4. The values of erosion rate constant k_e for various samples are listed in Table IV. The rate of poly-

Table II
Erosion of Copolyester Films after Hydrolytic Degradation in Aqueous Buffer at 55 °C and pH 7.4

		% of initial weight remaining at t (days)						
no.	sample	0	7	13	25	38	48	58
3	P(3HB-*co*-45% 3HV)	100	96	99	105	97	98	98
4	P(3HB-*co*-71% 3HV)	100	95	100	101	97	97	99
5	P(3HB-*co*-10% 4HB)	100	97	99	99	98	98	98
6	P(3HB-*co*-17% 4HB)	100	98	100	100	99	99	99
7	P(3HB-*co*-27% 4HB)	100	97	100	101	99	99	99

Table III
Molecular Weights of Copolyester Films after Hydrolytic Degradation in Aqueous Buffer at 55 °C and pH 7.4

time, days	no. 3, P(3HB-*co*-45% 3 HV)		no. 4, P(3HB-*co*-71% 3 HV)		no. 5, P(3HB-*co*-10% 4HB)		no. 6, P(3HB-*co*-17% 4HB)		no. 7, P(3HB-*co*-27% 4HB)	
	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_n \times 10^{-3}$	$\overline{M}_w/\overline{M}_n$
0	369	2.6	254	2.0	395	3.0	366	2.9	332	2.4
7	283	2.9	260	2.1	362	2.8	252	3.5	252	2.6
13	260	2.7	243	2.2	266	3.0	255	3.3	197	2.8
25	226	2.5	215	2.2	218	2.9	195	3.3	138	3.1
38	nd	nd	197	2.1	142	3.0	137	3.7	91	3.8
48	202	1.9	194	2.0	135	3.2	112	3.9	78	3.7
58	188	2.0	nd	nd	132	2.6	98	3.4	69	3.4

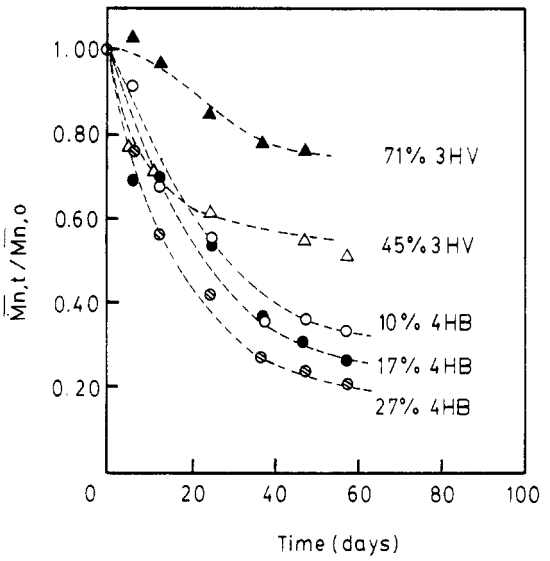


Figure 1. Changes in the $\overline{M}_n$ values of copolyester films during the hydrolytic degradation in a 0.01 M phosphate buffer at 55 °C and pH 7.4: (△) P(3HB-*co*-45% 3HV), sample 3; (▲) P(3HB-*co*-71% 3HV), sample 4; (○) P(3HB-*co*-10% 4HB), sample 5; (●) P(3HB-*co*-17% 4HB), sample 6; (○) P(3HB-*co*-27% 4HB), sample 7.

ester erosion by P(3HB) depolymerase is strongly dependent upon the compositions of polyesters and decreases in the following order: P(3HB-*co*-4HB) > P(3HB) > P(3HB-*co*-3HV), which is the same order with that (eq 1) observed in the hydrolytic degradation. As can be seen from the data of samples 1 ($\overline{M}_n$ = 768 000) and 2 ($\overline{M}_n$ = 22 000) in Figure 3, the erosion rate of P(3HB) film was essentially independent on the chain length of P(3HB).

Table V gives the molecular weight data of polyester films after enzymatic degradation. The $\overline{M}_n$ values of films remained almost unchanged during the course of enzymatic degradation. This result indicates that P(3HB) depolymerase hydrolyzes only the polyester chains in the surface layer of the film and polymer erosion proceeds via surface, not internal, dissolution.

The scanning electron micrographs (SEMs) of P(3HB) film (sample 1, $\overline{M}_n$ = 768 000) are shown in Figures 4 and 5. Figure 4 shows the SEMs of the cross-section (A) and surface (B) of virgin P(3HB) film (65 μm thick). Figure 5 shows the SEMs of the cross-section (A) and surface (B) of the P(3HB) film (22 μm thick) after an enzymatic degradation of 20 h. The weight of P(3HB) film decreased up to 32% during the enzymatic degradation, and the film thickness decreased from 65 to 22 μm (34% of initial thick), indicative of surface erosion by P(3HB) depolymerase. The surface of degraded P(3HB) film is apparently blemished by the function of depolymerase (see Figure 5B), while no change takes place in the inside of the film.

Table IV
Rate Constants k_d and k_e for Hydrolytic and Enzymatic Degradations of Microbial Polyester Samples

no.	sample	k_d,[a] day^{-1}	k_e,[b] mg h^{-1}
1	P(3HB), $\overline{M}_n$ = 768 000	nd	0.17 ± 0.03
2	P(3HB), $\overline{M}_n$ = 22 000	nd	0.15 ± 0.01
3	P(3HB-*co*-45% 3HV)	(4.5 ± 1.5) × 10^{-6}	0.03 ± 0.01
4	P(3HB-*co*-71% 3HV)	(2.3 ± 0.5) × 10^{-6}	0.04 ± 0.01
5	P(3HB-*co*-10% 4HB)	(8.3 ± 1.5) × 10^{-6}	0.80 ± 0.05
6	P(3HB-*co*-17% 4HB)	(11 ± 2) × 10^{-6}	0.90
7	P(3HB-*co*-27% 4HB)	(17 ± 2) × 10^{-6}	nd

[a] Rate constant for hydrolytic degradation (chain scission) at 55 °C. [b] Rate constant for enzymatic degradation (erosion) at 37 °C.

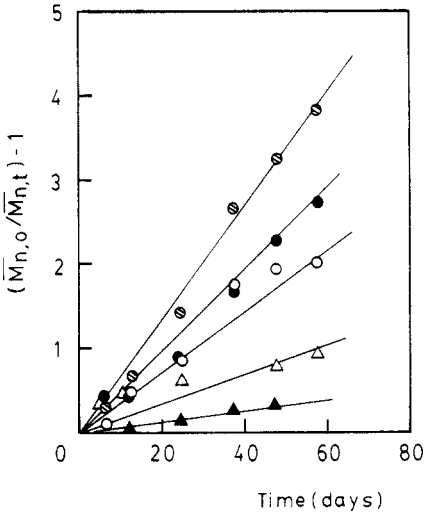


Figure 2. Plots of $[\overline{M}_n(0)/\overline{M}_n(t)] - 1$ versus time t for copolyester samples 3–7 (see caption of Figure 1).

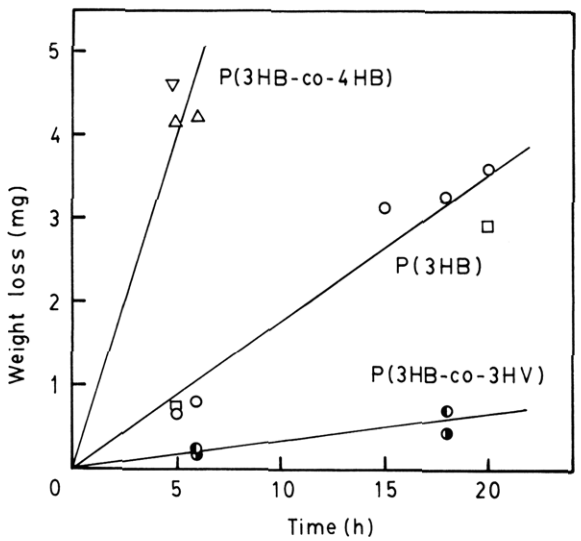


Figure 3. Enzymatic degradation (erosion) profiles on solution-cast films of six polyester samples in the aqueous solution of P(3HB) depolymerase at 37 °C and pH 7.5: (○) P(3HB) ($\overline{M}_n$ = 768 000), sample 1; (□) P(3HB) ($\overline{M}_n$ = 22 000), sample 2; (◑) P(3HB-*co*-45% 3HV), sample 3; (◐) P(3HB-*co*-71% 3HV), sample 4; (△) P(3HB-*co*-10% 4HB), sample 5; (▽) P(3HB-*co*-17% 4HB), sample 6.

Table V
Molecular Weight Distributions and Weight Loss of *Polyester Films after Enzymatic Degradation in Aqueous Solution of P(3HB) Depolymerase at 37 °C and pH 7.5*

no.	sample	time, h	initial wt remaining,[a] %	mol wt $\overline{M}_n \times 10^{-3}$	mol wt $\overline{M}_w/\overline{M}_n$
1	P(3HB)	0	100	768	1.9
1	P(3HB)	5	83	837	1.7
1	P(3HB)	20	32	669	1.9
2	P(3HB)	0	100	22	3.4
2	P(3HB)	20	42	28	3.3
5	P(3HB-*co*-10% 4HB)	0	100	395	3.0
5	P(3HB-*co*-10% 4HB)	5	36	352	2.5
6	P(3HB-*co*-17% 4HB)	0	100	366	2.9
6	P(3HB-*co*-17% 4HB)	5	43	331	2.6

[a] Initial weights of films (10 × 10 mm in size and 0.04–0.07 mm thick) = 5.0–8.0 mg.

For comparison, Figure 6 shows the SEMs of the P(3HB) film (75 μm thick) after a hydrolytic degradation of 48 days at 55 °C in a 0.01 M phosphate buffer (pH 7.4) without P(3HB) depolymerase. The weight of P(3HB) film was unchanged for 48 days, while the $\overline{M}_n$ value decreased from 768 000 to 245 000. The film thickness increased from 65 to 75 μm for 48 days, suggesting that water permeated the polymer matrix during the hydrolytic degradation. On the other hand, the surface of the film was apparently unchanged.

Conclusions

The hydrolytic degradation of microbial copolyester films occurred throughout the whole polymer matrix, and the molecular weights of copolyester films decreased with time in 0.01 M phosphate buffer at 55 °C and pH 7.4. However, the weights of all copolyester films were unchanged over 58 days. The molecular weight loss profiles of copolyester films by hydrolysis could be explained by the mechanism of a random chain scission of the ester groups. The rates of hydrolytic chain scission were dependent upon the compositions of copolyesters and decreased in the order of eq 1.

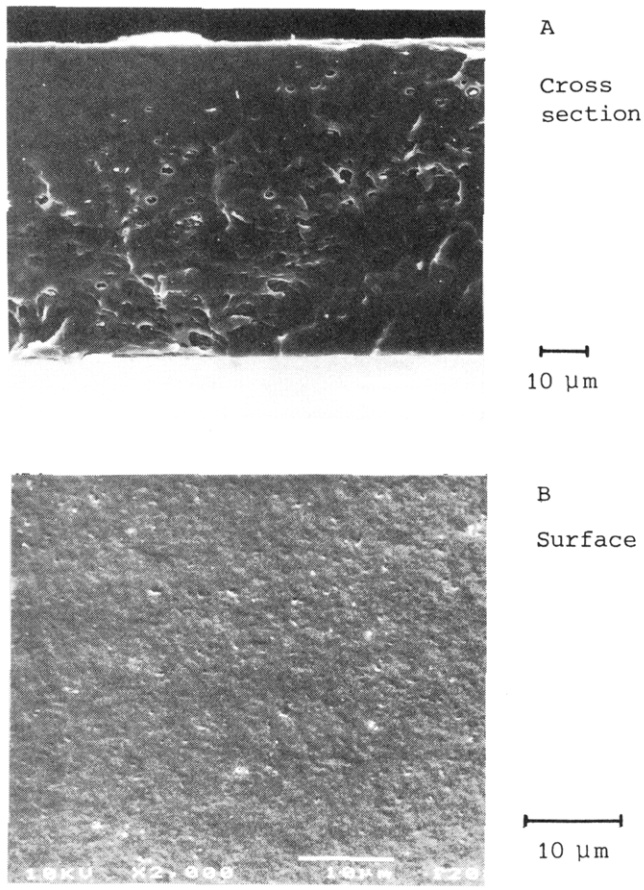


Figure 4. SEM of the cross-section (A) and surface (B) of virgin P(3HB) film (sample 1).

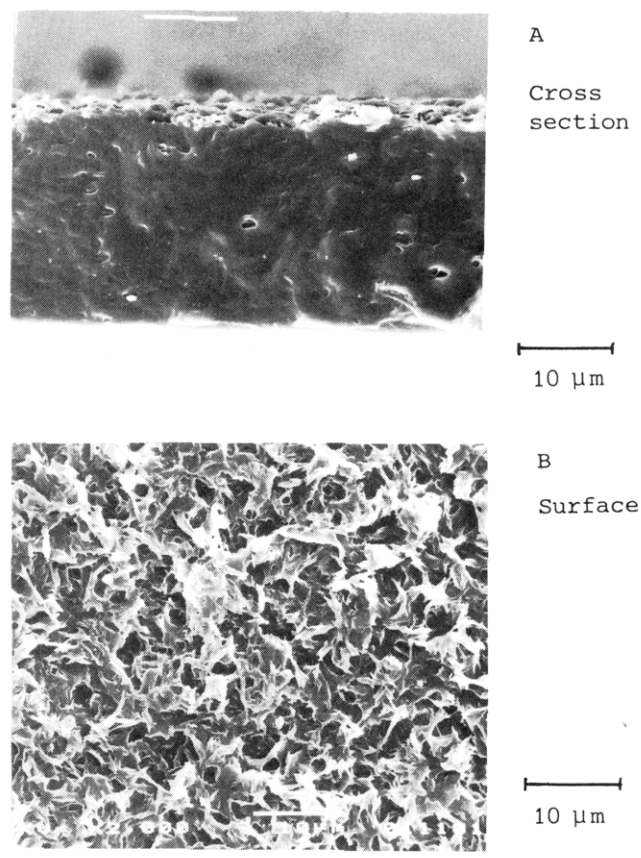


Figure 5. SEM of the cross-section (A) and surface (B) of the P(3HB) film after 20 h of enzymatic degradation at 37 °C.

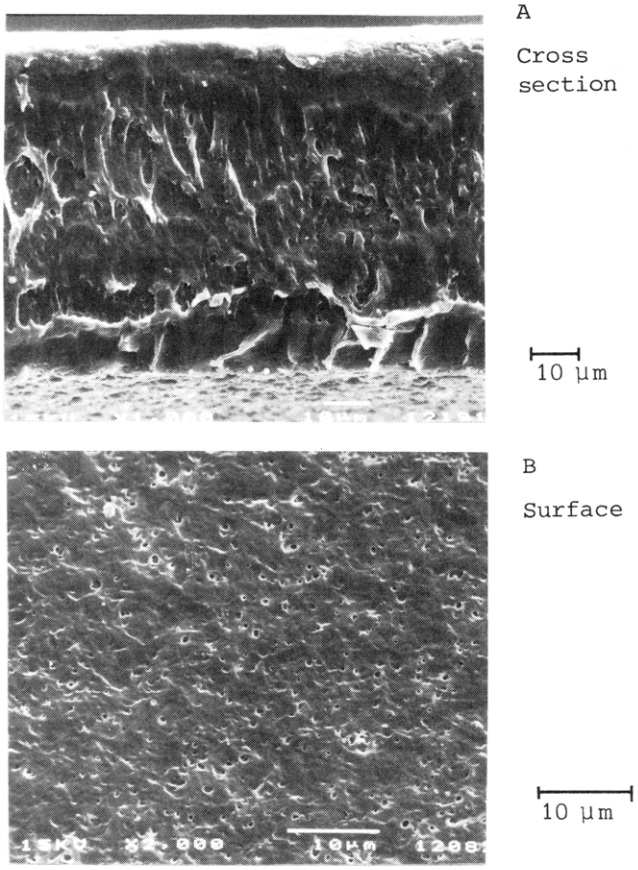


Figure 6. SEM of the cross-section (A) and surface (B) of the P(3HB) film after 48 days of hydrolytic degradation at 55 °C.

Miller et al.[28] and Holland et al.[29] reported the results of hydrolytic degradation of P(3HB) and P(3HB-*co*-3HV) (up to 20 mol % of 3HV) samples. Miller et al.[28] monitored the time-dependent changes in mechanical properties of the monofilament samples in a phosphate buffer (pH 7.2) at 60 and 70 °C and concluded that the presence of 3HV units retarded the degradation rate of monofilaments. In contrast, Holland et al.[29] monitored the time-dependent changes in polymer weight loss (erosion) rates of the solution cast films in aqueous solutions (pH = 2.3–10.6) at 37 and 70 °C and found that the presence of 3HV units enhanced the erosion rate. Holland et al. suggested that the enhancement of the hydrolytic degradation rate was due to a decrease in the crystallinity of copolyester film with increasing 3HV fraction. Bloembergen et al.[20] have pointed out that the crystallization of solution-cast films of P(3HB-*co*-3HV) is very slow (several weeks at room temperature to reach equilibrium crystallinity). In this study, we aged the solution-cast films for 3 weeks at room temperature prior to use and determined the degree of crystallinity of polyester films by X-ray diffraction. As shown in Table I, the crystallinities of P(3HB-*co*-3HV) films of various compositions are the same within experimental error. Therefore, we have concluded that the rate of hydrolytic degradation is not dependent upon the crystallinity of polyester film but upon the composition.

The enzymatic degradation processes of microbial polyester films were studied at 37 °C and pH 7.5 in the aqueous solution of the extracellular P(3HB) depolymerase from *A. faecalis*. The rate of enzymatic degradation on the respective copolyester films was much faster than the rate of simple hydrolytic degradation. The enzymatic degradation occurred at the surface of the polyester film, and the weight and thickness of film decreased with time. The molecular weights of polyester films remained almost unchanged during the enzymatic degradation. It has been found that the extracellular P(3HB) depolymerase hydrolyzes polyester chains in the surface layer of the film. Thus, polymer erosion proceeds via surface dissolution. The rate of polyester erosion by the depolymerase decreased in the order P(3HB-*co*-4HB) > P(3HB) > P(3HB-*co*-3HV), which is the same order with that observed in the simple hydrolytic degradation. Thus, the presence of 4HB units in polyesters accelerates the rates of both enzymatic and hydrolytic degradations. No correlation was found between the degradation rates and crystallinities of microbial polyester films. A rapid erosion of P(3HB-*co*-4HB) film by P(3HB) depolymerase may be due to a facile attack of the enzyme molecule toward the ester groups of copolyester chains, since the steric bulkiness of 4HB units is less than those of 3HB and 3HV units.

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Registry No. (3HB)(3HV) (copolymer), 80181-31-3; (3HB)(4HB) (copolymer), 117068-64-1.

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Thermal Isomerization Behaviors of a Spiropyran in Bilayers Immobilized with a Linear Polymer and a Smectitic Clay

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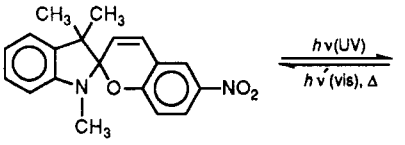
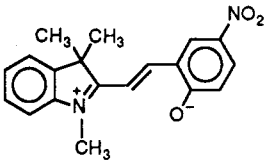
ABSTRACT: Thermal isomerization kinetics of photoinduced merocyanine to spiropyran is investigated in solid films having multibilayer structures, which consists of ion complexes between an ammonium bilayer forming amphiphile and polyanions. The reaction rates in these films abruptly increase near the crystal to liquid-crystal phase-transition temperature of the immobilized bilayer due to increased matrix mobility. The bilayer film immobilized with a smectitic silicate instead of a linear polymer provides more homogeneous reaction environments for the isomerizing chromophore and gives rise to a larger rate change at the phase-transition temperature. These improved properties by use of the clay is correlated to the increased order of bilayer structures in the film.

Introduction

The photochromic reaction of spiropyrans provides useful information on the dynamics of matrices as widely investigated in amorphous polymer solid films.[1] The reaction rate, which is frequently evaluated for the thermal reaction kinetics (isomerization of UV-induced merocyanine (PMC) → spiropyran (SP), scheme shown below),



SP (1)



PMC

is dependent on the mobility of the surroundings and, in polymer matrices, is influenced by the glass transition. The sensitivity of the reaction to matrices can be ascribed to large changes of the molecular shape between the isomers. Studies on photochromic reaction behaviors and their controls in solid media have potential significances for practical applications such as optical recording.

More recently matrix effects for this reaction have been explored in media having ordered structures such as thermotropic[2] and lyotropic[3] liquid crystals, bilayer membranes,[4-7] and Langmuir-Blodgett (LB) multilayers.[8-12] Studies in such ordered matrices have advantages that reaction behaviors may be understood from physicochemical properties on the molecular level. We have reported the photochromic behaviors of spiropyrans incorporated into ammonium-type bilayer membranes dispersed in water,[5-7] particularly focusing on the effect of the fluidity change, i.e., the crystal to liquid-crystal phase transition (the temperature, T_c).

Kunitake and his co-workers have proposed simple casting methods for immobilization of bilayers to obtain transparent thin solid films.[13-16] The cast films have multibilayered structures similar to those of Y-type films prepared in the LB technique,[15,16] and the films maintain the phase-transition behaviors of aqueous bilayers.[13-15,17,18] Our present attention is focused on the photochromic behavior of spiropyrans embedded in such solid bilayer-immobilized films. This paper describes the thermal isomerization behavior of a spiropyran **1** (in the scheme) embedded in cast bilayer films composed of ion complexes between dioctadecyldimethylammonium and polyanions. Our preliminary report has shown abrupt rate changes in the thermal reaction brought about by the crystal to liquid-crystal phase transition of the immobilized bilayer complexed with poly(styrene sulfonate) (PSS).[19] In this work polyanions of two different types are employed as "bilayer binders", a linear polymer (PSS) and a two-dimensional smectitic clay (montmorillonite, Mont). The complexation of the latter with organic cations is well-known and frequently referred to as intercalation.[20] It is found here that the type of the polyanion largely influences film structures and isomerization kinetics of **1** including the rate change at T_c. Effects of the phase transition on fundamental properties such as solute permeability and viscoelasticity of bilayer/PSS[17,18] and bilayer/Mont[21] films have been quite recently studied.

Experimental Section

Materials. 1,3,3-Trimethyl-6′-nitrospiro[indoline-2,2′-2′*H*-benzopyran) (**1**) was purchased from Tokyo Kasei Co. and recrystallized from ethanol. Dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1$) was obtained from Sogo Pharmaceutical Co. and recrystallized from ethyl acetate. Poly(styrene sulfonate) sodium salt (M_w = 50 000) was obtained from Scientific Polymer Product Co. and used without further purification. Montmorillonite used in this study was the product of Kurimine Ind.

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