Structural Effects upon Enzymatic Hydrolysis of Poly(butylene succinate-*co*-ethylene succinate)s

Masatsugu Mochizuki,* Katsuyuki Mukai, Kenji Yamada, Naoji Ichise, Shigemitsu Murase, and Yoshiaki Iwaya

Research and Development Center, Unitika Ltd., 23 Uji-Kozakura, Uji, Kyoto 611, Japan

Received January 14, 1997; Revised Manuscript Received July 1, 1997[⊗]

ABSTRACT: High molecular weight poly(butylene succinate-*co*-ethylene succinate)s, P(BS-*co*-ES)s, were obtained from succinic acid, 1,4-butanediol, and/or ethylene glycol through a polycondensation process. Enzymatic hydrolysis of hot-pressed copolyester films was carried out by utilizing lipases derived from a wide variety of microorganisms. The formation of water-soluble total organic carbon (TOC) was monitored to determine the effect of structure upon enzymatic degradability. The P(BS-*co*-ES) copolyester containing approximately 53 mol % ES exhibited a minimum in systemic crystallinity and a maximum in enzymatic degradation. These results suggest that the degree of crystallinity may be the dominant influence upon the rate of degradation. Furthermore, it appears that the sequence distribution of BS and ES subunits has little effect upon lipase activity.

Introduction

In recent years, the utility of biodegradable polymers[1] has received much attention due to their potential impact upon the complex issue of plastic waste management. Aliphatic polyesters are one of the most promising structural materials for biodegradable or compostable fibers, nonwovens, films, sheets, bottles, and injection-molded products. However, commercial use of high-molecular-weight aliphatic polyesters has been limited to polyesters produced by microorganisms,[2] ring-opening polymerization of lactones,[3] and ring-opening polyaddition of cyclic dimers.[4]

Much of this work has been generated due to the inherent difficulty in synthesizing high molecular weight aliphatic polyesters through the polycondensation of diols and dicarboxylic acids.[5] In response to this limitation, the use of chain-extending agents[6,7] as well as direct polycondensation in solvent[8] have proven successful in formulating high molecular weight aliphatic polysters. In addition, we have developed high molecular weight polyesters through a conventional polycondensation process without use of solvent as a combination of reaction conditions, effective catalysis, and heat stabilization is employed.[9]

Coinciding with this work, a considerable amount of qualitative and semiquantitative information has been accumulated in order to delineate the most important factors that affect the rate of degradation of solid polymers. Most notably, the enzymatic degradation of a solid polymer is influenced by the hydrophilic−hydrophobic balance within the main chain[10−14] coupled with its specific solid-state morphology.[15−17]

In the present study, we prepared high molecular weight aliphatic polyesters derived from a mixture of 1,4-butanediol, ethylene glycol, and succinic acid. The biodegradability of the resultant copolyesters by lipases originating from various kinds of microorganisms was investigated in order to understand the effects of structure upon enzymatic degradation.

Experimental Section

Synthesis of Aliphatic Polyesters. A three-necked flask equipped with an agitator, Vigreaux fractionation condenser, a thermometer, and a gas introduction tube was charged with 0.40 mol of succinic acid and 0.52 mol of 1,4-butanediol and/or ethylene glycol. The flask was immersed in a silicone oil bath and heated under nitrogen atmosphere for 3 h at 200 °C with water removed as the reaction byproduct of esterification. When water ceased to generate by condensation, 0.1 g of polyphosphoric acid, PPi, and 1.0×10^{-3} mol (per mole of succinic acid) of titanate, $Ti(OBu)_4$, or tetra-*n*-butoxygermanium, $Ge(Bu)_4$, as polycondensation catalyst were added to the vessel. Reaction was continued under reduced pressure of 0.5 mmHg at 220 and 240 °C for 1 h intervals, respectively.

The reaction scheme with chemical structure is as follows. Polycondensation was carried out first in an excess of glycol at normal pressure by an esterification reaction (eq 1), then with catalyst at high temperature under vacuum by deglycol reaction (eq 2).

$$\mathrm{HO(CH_2)_2OH + HO(CH_2)_4OH + HOOC(CH_2)_2COOH} \rightarrow \mathrm{H[O(CH_2)_4OOC(CH_2)_2CO]_m[O(CH_2)_2OOC(CH_2)_2CO]_n\text{-}O(CH_2)_2OH + 2(m+n)\,H_2O} \quad (1)$$

$$\rightarrow -\mathrm{[O(CH_2)_4OOC(CH_2)_2CO]_m[O(CH_2)_2OOC(CH_2)_2CO]_n-} + \mathrm{HO(CH_2)_2OH} \quad (2)$$

Composition of Copolyesters. The compositions of the P(BS-*co*-ES) copolyesters were determined by analyses of 300 MHz ^{1}H-NMR spectra recorded at 21 °C in $CDCl_3$ solution on a Varian VXR 300. The sequence distributions of BS and ES units were analyzed by 75 MHz ^{13}C-NMR spectra using Varian VXR 300.

Molecular Weight. Molecular weight data were obtained at 35 °C by gel permeation chromatography (GPC), using a Waters GPC device 600E (Detector: Waters 410 differential refractometer) with two ultrastyragel (pore size: 10^3−10^4 Å) columns. Chloroform was used as the eluent at a flow rate of 0.8 mL/min, and a sample concentration of 2.5 mg/mL was used. The number-average (M_n) and weight-average molecular weights (M_w) were calculated by using a calibration curve which was obtained by polystyrene standards with low polydispersity indicies. The reduced viscosity, η_{sp}/c, was measured by a Ubbelohde viscometer at a concentration of 0.5 g/dL in chloroform at 30 °C.

Thermal Properties. Differential scanning calorimetry (DSC) was administered to ∼10 mg samples under nitrogen flow on a Perkin-Elmer thermal analysis instrument (DSC-7). The melting temperature (T_m) and heats of fusion (ΔH_m) were determined from the initial heating scan from −100 to +200 °C at a rate of 20 °C/min. Following the initial scan, the samples were maintained at 200 °C for 1 min and rapidly quenched to −100 °C. The glass transition temperatures (T_g)

[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.

S0024-9297(97)00036-3 CCC: $14.00

Table 1. Characteristics of Synthesized Copolyesters

composition[a] (BS/ES)	polycondensation catalyst	heat stabilizer	η_{sp}/C (dL/g)	M_n	M_w/M_n	T_m (°C)	T_g (°C)	crystallinity[b] (%)
100/0 (100/0)	$Ti(OBu)_4$		1.32	59 000	2.5	117	−35	29.2
94/6 (90/10)	$Ti(OBu)_4$		1.22	59 000	2.4	109	−33	28.8
87/13 (80/20)	$Ti(OBu)_4$		1.10	50 000	2.8	102	−31	27.8
78/22 (70/30)	$Ti(OBu)_4$		1.12	51 000	3.1	92	−29	27.1
71/29 (60/40)	$Ti(OBu)_4$		1.10	49 000	2.9	83	−27	25.0
60/40 (50/50)	$Ge(OBu)_4$	PPi	1.20	53 000	3.0	64	−23	22.3
47/53 (40/60)	$Ge(OBu)_4$	PPi	0.96	48 000	2.5	47	−21	18.3
38/62 (30/70)	$Ge(OBu)_4$	PPi	0.86	42 000	2.5	54	−17	22.7
24/76 (20/80)	$Ge(OBu)_4$	PPi	0.77	35 000	3.0	66	−14	25.8
12/88 (10/90)	$Ge(OBu)_4$	PPi	0.90	43 000	2.6	88	−10	27.5
0/100 (0/100)	$Ge(OBu)_4$	PPi	1.12	53 000	2.0	104	−6	29.4

[a] Compositions of copolyesters were determined by ^{1}H-NMR, and the values in parenthesis were charge-based compositions. [b] Crystallinity of films was determined by X-ray method.

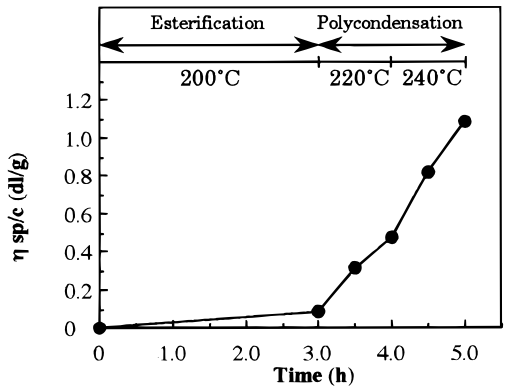

Figure 1. Time course of PES polymerization in the presence of tetra-*n*-butoxygermanium as a polycondensation catalyst and polyphosphoric acid as a heat stabilizer.

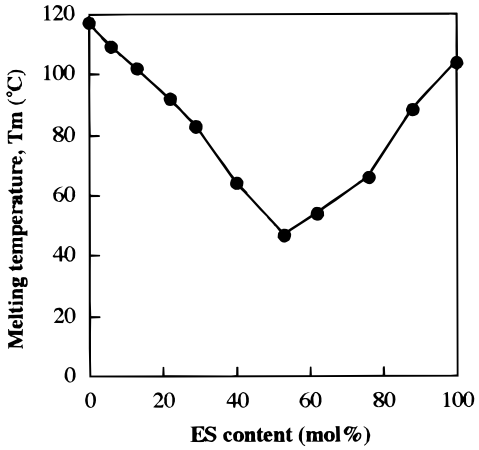

Figure 2. Change in melting point vs ES content in P(BS-*co*-ES).

were calculated as the midpoint of the heat capacity change as the samples were heated to 200 °C at 20 °C/min.

X-ray Analysis. The crystalline structure of hot-pressed films was analyzed by wide-angle X-ray diffraction (WAXD) using Cu Kα radiation with a RAD-γB (Rigaku Denki) operated at 50 kV and 200 mA.

The degree of crystallinity was measured with the aid of the Ruland method.[18]

Enzymatic Degradation. Reaction mixtures were formulated with the following constituents: (a) 10 × 10 mm hot-pressed copolyester film (thickness: 200−250 μm); (b) 20 mM phosphate buffer (pH 7.0); (c) 5 units/mL of lipase (Sigma Chemical) from *Rhizopus delemar*, *Rhizopus arrhizus*, *Phycomyces nitens*, *Aspergillus niger*, *Pseudomonas fluorescens*, or *Humicola lanuginosa*, with or without 0.005% Plysurf A210G (Daiichi Kogyo), and 5 mL of distilled water. One unit of activity was defined as that amount of enzyme which catalyzed the release of 1 μmol of free fatty acid from olive oil per minute at pH 7.0 and 30 °C. The reaction mixture was incubated in an Erlenmeyer flask on a rotary shaker at 80 rpm at 30 °C for 4 or 16 h. After incubation, the reaction mixtures were filtered through a Millipore filter (0.2 μm), and the water-soluble total organic carbon (TOC) formation in the reaction mixture was measured with a Shimadzu TOC-500.

Results and Discussion

Molecular Weight and Composition. Aliphatic polyesters with M_n ranging from 35 000 to 59 000 Da (Table 1) were obtained without coupling agents or chain extenders using the polycondensation process previously described. Figure 1 illustrates the typical time course of polycondensation reaction in terms of molecular weight. Following esterification, a sharp increase in $\eta_{sp}/_c$ is observed as the temperature is raised from 200 to 220 °C under reduced pressure as the deglycol reaction progresses. Furthermore, the molecular weight and composition was contingent upon the catalyst utilized. Higher molecular weights of PBS homopolymer and BS-rich P(BS-*co*-ES) were attained in the presence of $Ti(OBu)_4$. On the other hand, a combination of $Ge(OBu)_4$ and polyphosphoric acid yielded high molecular weights of PES homopolymer and ES-rich P(BS-*co*-ES). In each instance, the addition of polyphosphoric acid appeared to suppress thermal decomposition as molecular weight was enhanced and discoloration subsided.

In the course of polycondensation process, longer reaction time or higher reaction temperature is required because of a decrease in the number of reactive terminal groups as the polymerization progresses. It results in thermal decomposition prevailing over polymerization due to the poor heat stability of aliphatic polyesters. Therefore, a combination of a highly effective catalyst and a heat stabilizer with polymerization conditions that are not conductive to thermal decomposition is the key to the successful synthesis of high molecular weight aliphatic polyesters.

Thermal Properties. Table 1 outlines the thermal characteristics of films of PBS, PES, and P(BS-*co*-ES). In conjunction with ^{1}H-NMR analysis, the observed linear reduction in T_m (Figure 2) of the PBS homopolymer (117 °C) as ES units are introduced suggests that a random copolyester has been synthesized. Above 53 mol % ES, this theme continues as the T_m of PES homopolymer (104 °C) is approached. Similar results with regards to T_g (Figure 3) are seen throughout the P(BS-*co*-ES) compositional range as the T_g values of the copolymers increase linearly from the −35 °C of PBS to the −4 °C of PES, in accordance with the Fox equation.[19]

These suggest that the P(BS-*co*-ES) copolyesters have isodimorphism with the BS and ES units cocrystallizing, because the two monomer units have approximately the

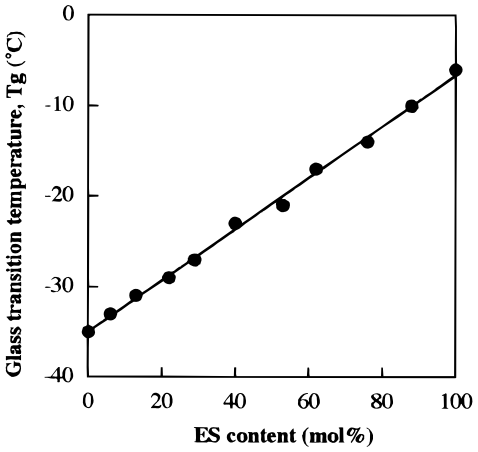

Figure 3. Change in glass transition temperature vs ES content in P(BS-*co*-ES).

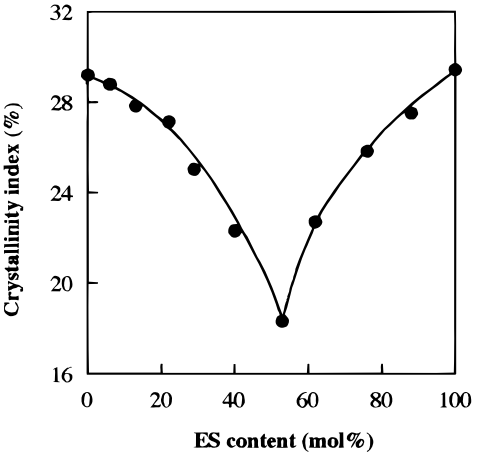

Figure 4. Change in X-ray crystallinity index vs ES content in P(BS-*co*-ES) films.

Table 2. TOC Formation of P(BS-*co*-ES) Films after 16 h in a 0.2 M Phosphate Buffer Solution in the Absence or Presence of Surfactant (0.005% Plysurf) Containing Lipases from Various Kinds of Microorganisms

	TOC formation (ppm)			
	absence of surfactant		presence of surfactant	
microorganism	BS/ES = 100/0	BS/ES = 78/22	BS/ES = 100/0	BS/ES = 78/22
R. delemar	0	58	26	82
R. arrhizus	8	440	46	374
P. nitens	0	731	0	87
A. niger	0	0	21	21
P. fluorescens	0	19	23	23
H. lanuginosa	0	0	0	0

same shape, similar volumes, and compatibility with each other. Below the melting point minimum, the ES unit is expected to crystallize in the BS lattice, and above the minimum, the BS unit is expected to crystallize in the PES lattice. It has been reported that the PBS molecules crystallize in the monoclinic crystal lattice,[20] and the crystal structure of PES has an orthorhombic unit cell.[21]

X-ray Analysis. X-ray studies revealed that the degree of crystallinity of the polyester films had a minimum value at around 53 mol % of the ES unit for the P(BS-co-ES) copolymers as shown in Figure 4.

Enzymatic Hydrolysis. Table 2 shows the results of enzymatic hydrolysis of P(BS-*co*-ES) films by lipases from various kinds of microorganisms in the absence and presence of surfactant. The effect of surfactant upon lipases except from *P. nitens* was minimal. In the case of *P. nitens* lipase, the addition of surfactant actually inhibited the enzymatic degradation.

Tokiwa et al.[22] added surfactant Plysurf A210G to the reaction mixture so that the hydrophobic surface of solid-state substrate might become susceptible to enzymatic attack in an aqueous phase. Additional effects of surfactant on enzymatic degradation, however, may depend upon the source of lipase. In case of *P. nitens*, it may prevent hydrophobic interactions between the hydrophobic substrate and the binding domain of the lipase.

It is generally accepted that aliphatic polyesters are susceptible to enzymatic attack compared to aromatic ones. Aliphatic polyesters are flexible enough to fit into the active sites of the enzymes, whereas aromatic polyesters are too rigid to permit this fit.[23] The enzymatic degradation of aliphatic polyesters depends upon not only the chemical structure as regards the hydrophilic−hydrophobic balance within the main chain but also the highly-ordered structure as regards its specific solid-state morphology and degree of crystallinity. Table 3 shows the results of the lipase activity with respect to a given polymeric composition of P(BS-*co*-ES). The effect of the BS/ES mole ratio within P(BS-*co*-ES) upon biodegradability by *R. arrhizus* lipase is shown in Figure 5. It is apparent that the hydrolysis rates of these copolymers were faster than those of each homopolymer with a maximum in TOC measured for 53 mol % ES. In the case of *P. nitens* lipase shown in Figure 6, there is a profile of enymatic hydrolysis activity similar to that of *R. arrhizus* lipase. As for what the major factor was that affected the enzymatic hydrolysis of P(BS-*co*-ES) copolymer films, it was concluded that the existence of their optimum mole ratio for biodegradation came from a mostly amorphous or less-ordered structure rather than the optimum chemical structure, because the profile of biodegradability vs polymeric composition is closely and inversely connected with the profiles of crystallinity vs polymeric composition. If the rate of enzymatic hydrolysis depends on the content of the BS or ES unit, the profile should have a tendency to show a linear relationship in the polymeric composition.

As for the dependence of molecular weight upon the rate of degradation, Fields et al.[10] have shown in their biodegradation studies on polycaprolactone that within the molecular weight range of 17 000−30 000, the rate of degradation is independent of the molecular weight. Tokiwa et al. also reported that M_n did not affect the rate of enzymatic hydrolysis by *R. arrhizus* lipase when M_n was more than 4000.[24] This would indicate that the lipases are endo-type enzymes that randomly split ester bonds in the polymer chains, and therefore in the polymers we studied, variations in the rate of degradation are not attributed to molecular weight differences but predominantly to structural differences.

Effects of Chemical Structure. Fields et al.[10,11] prepared polyesters from C_2−C_{12} diacids coupled with C_4−C_{12} dialcohols and found more microbial biodegradability of aliphatic polyesters from medium dialcohols/medium diacids than low dialcohols/low diacids or high dialcohols/high diacids, suggesting that the distance between ester groups was a factor in biodegradation. They concluded that the aliphatic polyesters most rapidly hydrolyzed were those made from moieties containing six carbon atoms, and increasing or decreas-

Table 3. TOC Formation of P(BS-*co*-ES) Films in the 0.2 M Phosphate Buffer Solution Containing Lipases from *R. arrhizus* and *P. nitrens*

	TOC formation (ppm)										
microorganism	BS/ES = 100/0	BS/ES = 94/6	BS/ES = 87/13	BS/ES = 78/22	BS/ES = 71/29	BS/ES = 60/40	BS/ES = 47/53	BS/ES = 38/62	BS/ES = 24/76	BS/ES = 12/88	BS/ES = 0/100
R. arrhizus (without surfactant, 16 h)	8	22	100	440	462	667	727	684	868	414	48
R. arrhizus (with surfactant, 16 h)	46	118	179	374	636	459	524	616	202	134	51
P. nitens (without surfactant, 4 h)	0	0	0	201	349	1,239	1,669	840	224	32	0

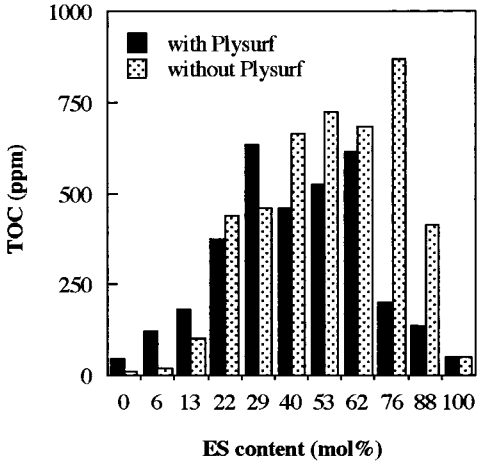

Figure 5. TOC formation profiles of P(BS-*co*-ES) films after 16 h in the aqueous solution containing lipase of *R. arrhizus* at 30 °C.

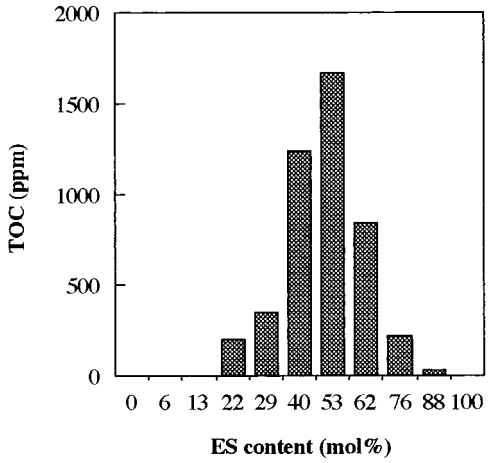

Figure 6. TOC formation profile of P(BS-*co*-ES) films after 4 h in the aqueous solution containing lipase of *P. nitens* at 30 °C.

ing the spacing between ester groups made the polymers less susceptible to enzymatic attack.

Diamond et al.[12] examined eight polyester films derived from C_2-C_{10} α,ω-aliphatic diols and C_4-C_{10} dicarboxylic acids to determine differences in biodegradability. The relative degradability of the polymers examined seemed to be related to the carbon chain spacing between ester groups. Longer chain polyesters exhibited only a slight degradation in both the agar culture test and the soil burial test.

Bitritto et al.[13] studied a series of poly(alkylene tartrate)s with similar results that they were in agreement with those of Fields et al.,[10,11] which postulated that the biodegradability of a polymer was affected by the hydrophilicity−hydrophobicity balance of the polymer.

Mukai et al.[14] investigated the enzymatic degradation of five different polyhydroxyalkanoate films at 37 °C in the aqueous solutions containing different microbial enzymes of 16 lipases and found that the number of lipases capable of hydrolyzing PHA chains decreased with an increase in the number of the methylene carbon atoms of the monomeric unit.

However, it seems that these studies basically lack the solid-state morphological point of view, because the degree of crystallinity of the polymers was not necessarily evaluated. One has to wonder if some property such as a difference in the crystallinity of the polymers could explain the difference in biodegradability.

Enzymes can be either totally specific for a given substrate, to such an extent that they will not tolerate any structural or configurational changes in the substrate, or they can be broadly specific for a given type of functional group, and they will still operate on substrate with structural variations around the functional group. Esterases, the enzymes that hydrolyze polyesters, are known to be low in specificity. They will hydrolyze a large variety of substrates, although not all at the same rate. One example of an esterase is lipase, which can catalyze the hydrolysis of many different types of both fatty acid esters of glycerol (triacyl glycerides) and aliphatic polyesters.[14,25] This coincides with the fact that lipases could not recognize the differences between BS and ES subunits within the main chain in the present study.

Effects of Highly-Ordered Structure. The effects of solid-state morphology on the course of biodegradation of semicrystalline polymers have been shown first by Huang and co-workers in their biodegradation study of polycaprolactone.[15,16] They have shown that the biodegradation proceeded more preferentially in amorphous regions than in crystalline regions.

We have recently observed very similar effects with them in our quantitative investigation of enzymatic degradation of polycaprolactone fibers with different draw ratios.[17] It was observed that the enzymatic degradation of the fibers as monitored by TOC formation and weight loss decreased with an increase in draw ratio. The surface erosion mechanism was supported by SEM observations of a gradual decrease in fiber diameter with enzymatic degradation time. The rate-determining factor of the enzymatic degradation appeared to be closely related to the content of crystalline and highly-ordered regions, since even a relatively small difference in the crystallinity of the fibers with the same primary structure strongly affected the rate of enzymatic degradation.

In the present study, the sequence distribution of BS and ES subunits within the main chain had little effect upon lipase activity, while the crystallinity profile was closely related to the lipase activity. Therefore it is concluded that the major factors affecting the rate of degradation of P(BS-*co*-ES) copolymer may be the solid-state morphology due to the crystallinity and the highly-ordered structure rather than the primary structure.

In order to explore the structural effects on the rate of degradation, Fields et al.[10,11] investigated the depen-

dence of weight loss, which was measured after 21 days at 30 °C with an inoculation of *Pullularia pullulans*, on polymeric composition for copolymers made from 1,4-butanediol and a mixture of adipic and sebacic acids, and they found that the rates of degradation for the copolymers were faster than the rates for the two homopolymers.[9,10] This would suggest that there was more amorphous or less-ordered regions in the copolymers, although they did not measure the degrees of crystallinity of the polymers.

The enzymatic degradation of (*R*)-3-hydroxybutyrate and 4-hydroxybutyrate, P(3HB-*co*-4HB), films were carried out at 37 °C in a 0.1 M phosphate buffer (pH 7.4) containing the extracellular depolymerase purified from *Alcaligenes faecalis* by Doi et al.[26,27] They reported that the rate of enzymatic degradation of the films increased with a decrease in the crystallinity.

Mechanism of Enzymatic Degradation. The enzymatic degradation of insoluble substrate by enzymes takes place via surface erosion.[15,17] The kinetics and mechanism of heterogeneous hydrolysis on the surface of poly[(*R*)-3-hydroxybutyrate] film have been studied using three types of extracellular poly(hydroxyalkanoate) (PHA) depolymerases by Mukai et al.[28] The kinetic data were accounted for in terms of heterogeneous enzymatic reaction via two steps of adsorption and hydrolysis by PHA depolymerases. The kinetic results suggest that the properties of the catalytic domains as an active site are very similar among the three PHA depolymerases but that those of the binding domains[29,30] adhering to the hydrophobic substrate are strongly dependent on the types of depolymerase.[31] A very high hydrophobicity of the enzyme is essential for adherence to the hydrophobic surface of substrate in aqueous environments.

In this paper, as can be seen in Table 2, the enzymatic degradation of P(BS-*co*-ES) by the lipases is highly dependent upon the lipase source. Among them, *P. nitens* lipase had the strongest activity upon the P(BS-*co*-ES) films although the lipase activity was reduced by the addition of the surfactant. The dependence of the enzymatic degradation upon the source of lipase may be attributed to the differences of the structures and hydrophobic properties of the binding domains rather than the catalytic domains of lipases, because it had been found out that all lipases had very similar structures of the catalytic domains with a common amino acid sequence, $-Gly-X_1-Ser-X_2-Gly-$, around the active center.[32]

References and Notes

(1) Lenz, R. W. *Adv. Polym. Sci.* **1993**, *107*, 1.
(2) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990.
(3) Cox, E. F.; Hostettler, F.; Charleston, W. V. U.S. Patent, 3 021 309, 1962.
(4) Schneider, A. K. U.S. Patent, 2 703 316, 1955.
(5) Carothers, W. H.; Hill, J. W. *J. Am. Chem. Soc.* **1932**, *54*, 1579.
(6) Takiyama, E.; Niikura, I.; Hatano, Y. U.S. Patent. 5 306 787, 1993.
(7) Takiyama, E.; Fujimaki, T. In *Biodegradable Plastics and Polymers*; Doi, Y., Fukuda, K., Eds.; Elsevier Science B. V.: Amsterdam, The Netherlands, 1994; p 150.
(8) Ajioka, M.; Enomoto, K.; Suzuki, K.; Yamaguchi, A. *Bull. Chem. Soc. Jpn. A* **1995**, *68*, 2125.
(9) Iwaya, Y.; Mukai, K.; Kawanishi, M.; Nishinohara, M. U.S. Patent. 5 504 148, 1996.
(10) Fields, R. D.; Rodriguez, F.; Finn, R. K. *J. Appl. Polym. Sci.* **1974**, *18*, 3571.
(11) Fields, R. D.; Rodriguez, F. In *Proceedings of the Third International Biodegradation Symposium*; Sharpley, J. M., Kaplan, A. M., Eds.; Applied Science: Barking, England, 1976; p 775.
(12) Diamond, M. J.; Feedman, B.; Garibaldi, J. A. *J. Int. Biodeg. Bull.* **1975**, *11*, 127.
(13) Bitritto, M. M.; Bell, J. P.; Brenckle, G. M.; Huang, S. J.; Knox, J. R. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1979**, *35*, 405.
(14) Mukai, K.; Doi, Y.; Sema, Y.; Tomita, K. *Biotech. Lett.* **1993**, *15*, 601.
(15) Jarrett, P.; Huang, S. J.; Bell, J. P.; Cameron, J. A.; Benedict, C. *Org. Coat Appl. Polym. Sci. Proc.* **1982**, *47*, 45.
(16) Huang, J. C.; Shetty, A. S.; Wang, M. S. *Adv. Polym. Technol.* **1990**, *19*, 23.
(17) Mochizuki, M.; Hirano, M.; Kanmuri, Y.; Kudo, K.; Tokiwa, Y. *J. Appl. Polym. Sci.* **1995**, *55*, 289.
(18) Ruland, W. *Acta Crystallogr.* **1961**, *14*, 1180.
(19) Fox, T. G. *Bull. Am. Phys. Soc.* **1956**, *1*, 123.
(20) Ihn, K. J.; Yoo, E. S.; Inn, S. S. *Macromolecules* **1995**, *28*, 2460.
(21) Ueda, A. S.; Chatani, Y.; Tadokoro, H. *Polym. J.* **1971**, *2*, 387.
(22) Tokiwa, Y.; Suzuki, T. *Agric. Biol. Chem.* **1978**, *42* (5), 1071.
(23) Huang, S. J. *Encycl. Polym. Sci. Eng.* **1985**, *2*, 220.
(24) Tokiwa, Y.; Suzuki, T. *Agric. Biol. Chem.* **1988**, *52*(8), 1937.
(25) Tokiwa, Y.; Suzuki, T. *Nature* **1977**, *270*, 76.
(26) Nakamura, S.; Doi, Y.; Scandola, M. *Macromolecules* **1992**, *25*, 4237.
(27) Saito, Y.; Doi, Y. *Biodegradable Plastics and Polymers*; Doi, Y., Fukuda, K., Eds.; Elesevier Science B. V.: Amsterdam, The Netherlands, 1994; p 394.
(28) Mukai, K.; Yamada, K.; Doi, Y. *Int. J. Biol. Macromol.* **1993**, *15*, 361.
(29) Shirakura, Y.; Fukui, T.; Saito, T.; Okamoto, Y.; Narikawa, T.; Koide, T.; Tomita, K.; Takemasa, T. *Biochim. Biophys. Acta* **1986**, *880*, 46.
(30) Saito, T.; Iwata, A.; Watanabe, T. J. *Environ. Polym. Degrad.* **1993**, *1*, 99.
(31) Mukai, K.; Yamada, K.; Doi, Y. *Polym. Degrad. Stab.* **1993**, *41*, 85.
(32) Boel, E.; Huge-Jensen, B.; Christensen, M.; Thim, L.; Fill, N. P. *Lipids* **1988**, *23*, 701.

MA970036K