Polymer Journal, Vol. 29, No. 10, pp 836—842 (1997)

The Morphology and Enzymatic Degradation of Chain-Extended Copoly(succinic anhydride/ethylene oxide) Films

Yasukatsu MAEDA,† Atsuyoshi NAKAYAMA, Norioki KAWASAKI, Kazuko HAYASHI, Seiichi AIBA, and Noboru YAMAMOTO

Department of Organic Materials, Osaka National Research Institute, AIST, 1-8-31 Midorigaoka, Ikeda, Osaka 563, Japan

(Received February 24, 1997)

ABSTRACT: The enzymatic degradation of the chain-extended copoly[succinic anhydride (SA)/ethylene oxide (EO)], synthesized by the ring-opening copolymerization, was studied using the lipase from *Rhizopus arrhizus*. A decrease in SA content of these copolymers resulted in substantial increase of their degradability. The lipase concentration was shown to enhance considerably the degradation of the copolymer film (polymer composition SA/EO = 42/58 mol%) which exhibited the highest susceptibility to degradation. The water contact angle measurements conducted on the films and their water absorption were related to the easy of adsorption of the lipase to the surface of the copolymer film and were not found to be particularly influenced by SA content. The degrees of crystallinity of the copolymer films, determined from wide angle X-ray diffraction, were within a range 24 to 28% as SA content increased from 42 to 48 mol%. The small angle X-ray scattering profiles indicated that these copolymer films had alternating crystalline layer structure. The long-period between lamellar crystalline, calculated from scattering angle, was found to increase from 80 to 110 Å with a decrease in SA content. The long-period, related to the size of amorphous region, was shown to affect the enzymatic degradation of copolymer by the lipase.

KEY WORDS Biodegradability / Polyethylenesuccinate / Enzymatic Degradation / Wide Angle X-Ray Diffraction / Small Angle X-Ray Scattering / Crystallinity /

Biodegradable polymers have been widely investigated from a viewpoint of global environmental preservation.[1-10] Aliphatic polyesters, which are typically biodegradable, have been prepared by two synthetic routes, namely biosynthesis[11-15] and chemical synthesis.[16-34] Biodegradation of the aliphatic polyesters was confirmed by using enzymes and microorganisms. However, it is noteworthy that there are only a few reports[35-37] referring to the relationship between morphology, such as crystal structure and higher-order structure, and biodegradability of the aliphatic polyesters except for the case of poly(3-hydroxybutylate) (PHB). Holland *et al.*[38] examined the effect of sample preparation technique on hydrolytic degradation and showed that various forms of PHB and copolyesters show different stabilities to hydrolytic attack. Doi *et al.*[39,40] investigated the crystalline structure and enzymatic degradation behavior of PHB and its copolyesters, and reported that the chemical composition of copolyesters affected the enzymatic degradability more than the crystallinity. Tokiwa *et al.*[41] studied the effects of higher-order structure of PHB on microbial degradation and the isolated microbial degradation was found to occur according to, at least, two different mechanisms. One was preferential degradation of amorphous phase and the other was non-preferential spherical degradation occurring on the film surface. Doi *et al.*[42,43] proposed a model of enzymatic hydrolysis of PHB by PHB depolymerase and suggested that the binding domain of depolymerase adheres selectively to the PHB crystalline phase on the surface of PHB film and that the catalytic domain hydrolyzes predominantly PHB chains in the amorphous phase on the surface and subsequently erodes PHB chains in the crystalline phase. The biodegradation behavior of PHB have been extensively studied by many workers.[44-46] These publications mainly reported on the effect of depolymerase on PHB films comprised of crystalline phase and amorphous phase. However, to the best of our knowledge there has been no publication reporting on the effects of the degree of crystallinity and morphology, crystalline and amorphous phase, on the enzymatic degradation of biodegradable polymers. It is thought that higher-order structure in polymers can play an important role toward controlling their biodegradability.

We have been recently investigating the chain-extension reaction of copoly[succinic anhydride (SA)/ethylene oxide (EO)], synthesized by the ring-opening copolymerization of SA and EO, for preparing high molecular weight copolyesters or copolyesterethers.[47] The relationship between the biodegradability and the thermal properties of the copoly(SA/EO)s having different SA molar content and different forms such as powder, bulk flake, and film was reported as well.[48] Among them, it has become apparent that the degradation by enzymes and by activated sludge of these copoly(SA/EO)s substantially decreased with an increase in SA molar content. Differential scanning calorimetry (DSC) analysis showed that the melting point of these copolymers rose remarkably with the increase in the SA molar content.[47,48]

In this work, we aim first to clarify the higher-order structure of the chain-extended copoly(SA/EO)s by wide angle X-ray diffraction (WAXD) and small angle X-ray scattering (SAXS) and to discuss the influence of higher-order structure of the copolymers on the enzymatic degradation behavior.

† To whom correspondence should be addressed (Tel: +81-727-51-9522; Fax: +81-727-51-9628).

EXPERIMENTAL

Materials

SA (from Wako Pure Chemical Co., Japan) was recrystallized from chloroform. EO (from Sumitomo Seika Co., Japan) was distilled over CaH_2 under reduced pressure. Initiators for the ring-opening copolymerization and titanium *iso*-propoxide (TIP) as a catalyst for the chain extension reaction were used as purchased from Wako Pure Chemical Co., Japan. The enzyme for biodegradation tests was the lipase from *Rhizopus arrhizus* of Boehringer Mannheim.

Synthesis of Copoly(succinic anhydride/ethylene oxide)

A series of copoly(SA/EO)s were prepared by a ring-opening copolymerization technique described elsewhere.[48] A representative example of a chain-extension reaction of copoly(SA/EO)s is as follows: the copolymer (3 g) was placed in a 100 ml three-necked flask equipped with a stirrer. After the copolymer was molten by heating, TIP (0.025 g) was added and kept stirring under vacuum at 170°C for 4—12 h. Polymerization products were dissolved in chloroform and the soluble materials were separated by filtration. The chloroform solution was concentrated *in vacuo*. The polymers were precipitated from the chloroform solution with petroleum ether and dried under vacuum at 80°C for 24 h. The synthesized chain-extended copoly(SA/EO)s are listed in Table I.

Preparation of Films

Film specimens (thickness: 50—100μm) were formed by compression molding of polymer powder or flake a laboratory press at the melting temperature of the copolymers for 30 s at 50 kg cm^{-2}. The prepared films were aged for at least 7 days at room temperature to reach equilibrium crystallinity.

Enzymatic Degradation

Enzymatic hydrolysis tests were carried out as follows: Twenty five mg of polymer samples and 2ml of phosphate buffer (KH_2PO_4/Na_2HPO_4, pH 7.0) were placed in a test tube, and the prescribed units of enzyme were added. Blank tests were conducted both for the polymers suspended without enzyme and for enzyme itself. The enzyme used was the lipase from *Rhizopus arrhizus*. The enzymatic hydrolysis tests were carried out at 37°C for the fixed time. After filtration (0.2μm membrane filter), total organic carbon (TOC) of the filtrate was measured in duplicate. The TOC data were averaged and corrected appropriately by taking into account the blank levels. Enzymatic degradation was also determined by weight loss measurement. The films (film dimensions: 15 × 20 mm and *ca.* 50μm thick) were placed in small bottles containing 3.0 ml of phosphate buffer. The lipase from *Rhizopus arrhizus* was added and the solutions were incubated at 37°C for 24 h. The films were periodically removed, washed with water, and dried to constant weight *in vacuo* before analysis.

Analytical Procedures

Characterization of Copolymers. ^{1}H NMR spectra were recorded on a JEOL JNM A-500 spectrometer (500 MHz). All spectra were obtained from chloroform-*d* solutions at room temperature with TMS as an internal standard. IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. Films were cast on a potassium bromide plate from chloroform solutions. Differential scanning calorimetry (DSC) studies were conducted with a Seiko Denshi DSC120 using the samples from 4 to 8 mg , at a heating rate of 10°C min^{-1} within a temperature range from −60 to 120°C (first scan). After first scan, the sample was cooled to −60°C at a rate of *ca.* 10°C min^{-1}, followed by second scan under the same conditions. Molecular weights (M_n) and molecular weight distributions (M_w/M_n) were determined by GPC (Tosoh, HCL-8020). The columns were a TSKgel G4000HXL and a TSKgel G3000HXL with limited exclusion molecular weight of 4×10^5. Chloroform was used as an eluent at a flow rate of 0.6 ml min^{-1}. Polystyrene standards with low polydispersities were used to generate a calibration curve. Contact angles of deionized water on the surface of the polymer films were measured at room temperature by the sessile drop method using a contact anglemeter (Kyowa Kaimenkagaku Co., Ltd., CA-A). Water absorption (wt%) of polymer films was calculated according to eq 1. Weight A of films after drying by vacuum for 24 h at 30°C and weight B of wet films dipping in deionized water for 24 h at 37°C were measured (Water on film surface was wiped by a filter paper).

$$\text{Water absorption } (\%) = 100(B-A)/A \qquad (1)$$

The X-ray measurements of polymer films (50—100 μm) were carried out with a Rigaku RINT 2500 (40 kV and 30 mA) at room temperature using a nickel-filtered Cu-K_α source. The WAXD measurements were carried out with point focusing. The intensity distribution was measured in the 2θ range 2°—140° at a scan speed of 4.0° min^{-1}. Degrees of crystallinity of copolymer films were calculated from diffracted intensity data according to Ruland's method.[49] SAXS measurements were performed on a small angle scattering goniometer with an 18 kW Rigaku Co. rotating anode X-ray generator operated at 50 kV and 300 mA at room temperature. (Rigaku RINT-2500, Nickel-filtered Cu-K_α radiation ($\lambda = 0.1542$ nm)).

Table I. Comonomer composition and molecular weight distribution of chain-extended copoly[succinic anhydride (SA)/ethylene oxide (EO)]s

Specimen	Polym. comp.[a] SA/EO (mol%)	M_n[b]	M_w/M_n[b]
1	41/59	38200	1.8
2	42/58	47000	1.7
3	43/57	48900	1.7
4	44/56	54300	1.9
5	47/53	36300	1.6
6	48/52	34700	1.6
7	49/51	38200	1.6

[a] Determined by ^{1}H NMR. [b] Determined by GPC.

RESULTS AND DISCUSSION

Enzymatic Degradation

The biodegradation of copoly(SA/EO)s by lipase from *Rhizopus arrhizus* was previously found to increase with a decrease in SA content of their copolymers.[47,48] In order to study the enzymatic degradation behavior, the degradation tests on biodegradable copoly(SA/EO) [SA/EO = 42/58 mol% (M_n = 47000)] were carried out by varying enzymatic activity or erosion time. Figure 1 shows the relationship between enzymatic activity of the lipase from *Rhizopus arrhizus* and degradability determined by weight-loss and TOC measurements of water-soluble compounds produced by enzymatic hydrolysis. In the case of using more than 3200U of the lipase, the value of TOC went up to theoretical value (*ca.* 6500 ppm) determined from their chemical structural formula and weight-loss reached around 100% within 15 h. The semicrystalline copolymer was almost hydrolyzed by the lipase from *Rhizopus arrhizus*. Below 3200U of the lipase, a linear relationship existed between the enzymatic degradation and enzymatic activity. As mentioned previously, Doi *et al.*.[42,43] reported that the enzymatic degradation on the surface of PHB film by the PHB depolymerase of *A. faecalis*, takes place *via* a two step mechanism; adsorption and hydrolysis. The first step consists of adsorbing the enzyme on the surface of PHB film *via* the binding domain, and the second step is hydrolysis of the polymer chain by the catalytic site. Under higher enzyme concentration, the hydrolysis rate decreases due to longer distance between the active site of the PHB depolymerase and the polymer chains in amorphous region. On the contrary, in the case of the enzymatic hydrolysis of chain-extended copoly(SA/EO) films by lipase from *Rhizopus arrhizus*, the above mentioned enzymatic degradation behavior of PHB was not observed. This result indicates that the biodegradation rate of copoly(SA/EO) film within 30000 U of lipase differs from the one of PHB by the PHB depolymerase because the lipase easily hydrolyzes the crystalline phase in the copoly(SA/EO) film.

Figure 2 shows the enzymatic degradation of copolymer films of same composition, as used in Figure 1, *versus* erosion time using 400U, 1600U of the lipase. Without addition of enzyme, the recorded TOC value was that of blank level and its value did not change even after long time (7 days). It is thought that the water-soluble oligomers in the copolymer film eluted into the the buffer solution. By addition of 1600U of lipase, there was an approximately linear relationship between the enzyme degradation and erosion time up to 10 h. The enzymatic degradation appeared to level off at erosion times higher than 10 h. The weight-loss of the film by enzymatic hydrolysis was about 3 times as that of 400U lipase for 20 h. The results from Figures 1 and 2 suggest that the lipase preferably attacks the degradable domain (amorphous phase) and the enzymatic hydrolysis rates decrease with time. Furthermore, these results were consistent with the degradation behavior of the PHB film by the PHB depolymerase reported by Doi and coworkers.[42,43] Acidification of the hydrolysis solution because of acidic compound produced by enzymatic hydrolysis will cause inactivation of the enzyme. The

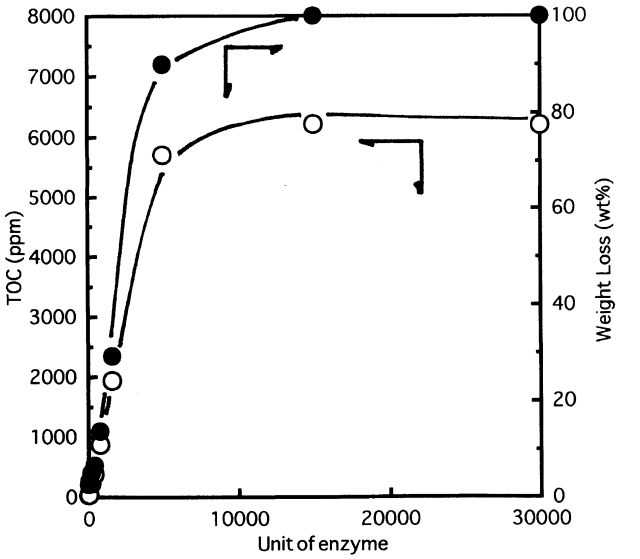

Figure 1. Enzymatic hydrolysis of the extended copoly[succinic anhydride (SA)/ethylene oxide (EO)] film (polym. comp., SA/EO = 41/59, M_n = 32800, film dimension, 15 × 20 mm in size, and *ca.* 50 μm thick) *versus* amount of enzyme units in buffer solution (pH = 7.0, 2 ml) for 15 h at 37°C. (○) total organic carbon (TOC) of water-soluble compounds produced by enzymatic hydrolysis; (●) weight-loss of the polymer films.

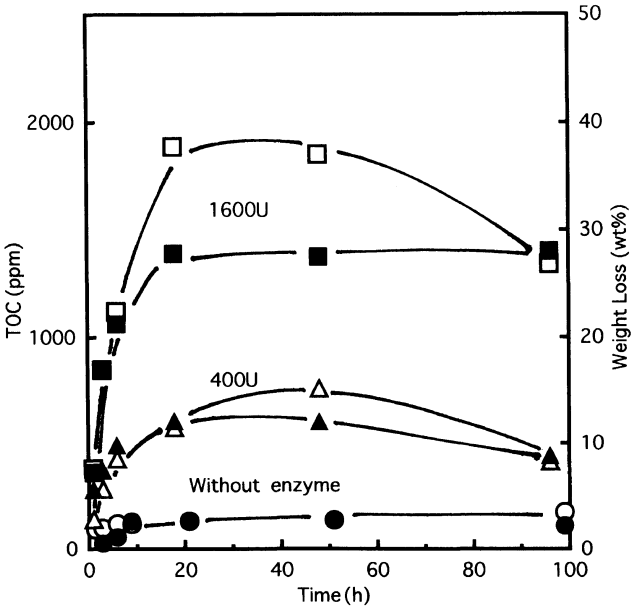

Figure 2. Enzyme hydrolysis rate *versus* amount of enzymatic units. (○, △, □) total organic carbon of water-soluble compounds produced by enzymatic hydrolysis, (●, ▲, ■) weight-loss of the polymer films.

pH of the hydrolysis solution reached 6.0 in the case of 30 wt% weight-loss. Desuelle *et al.*[50] reported that the optimum performance of the lipase from *Rhizopus arrhizus* was at pH 8.0 and 37°C. After 50 h of erosion time, it was found that TOC value, quite surprisingly, decreased with erosion time although the weight-loss remained constant. The reason of this phenomenon has not been clarified yet and further investigation is required.

Factors Affecting the Enzymatic Degradation

Figure 3 shows the relationships between the weight-loss by enzymatic degradation and the melting tem-

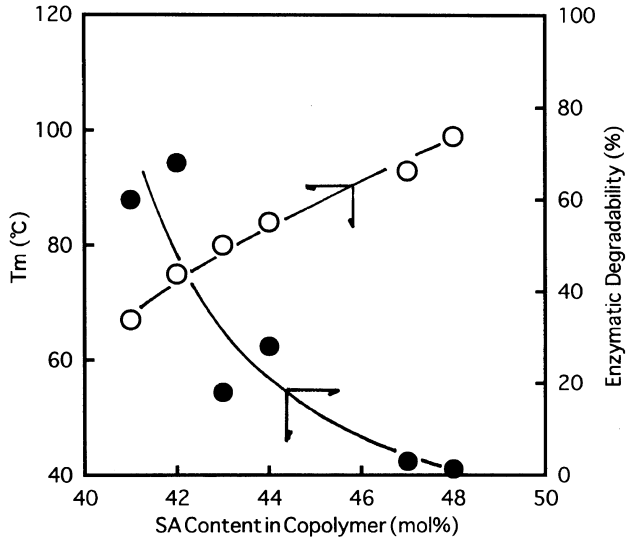

Figure 3. Melting point, T_m (○) or enzymatic degradation (●) against SA content in the extended copoly(SA/EO) films.

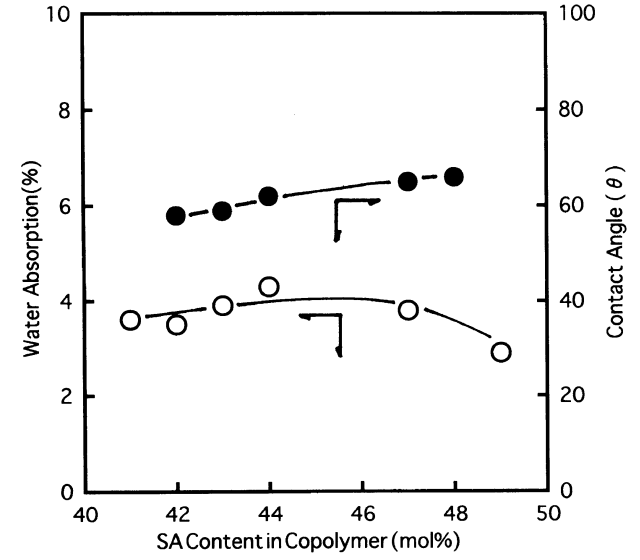

Figure 4. The water absorption (○) and the contact angle of water on the surface of copolymer films (●) *versus* SA content in the extended copoly(SA/EO) films.

perature (T_m) *versus* the SA content in the extended copoly(SA/EO) films. The enzymatic degradability drastically decreased with an increase in SA content of these copolymers. This result was in agreement with the previously reported data[48] obtained from TOC measurements for the water-soluble oligomers produced by enzymatic hydrolysis. The T_m of these copolymer films rose from 60 to 100°C as SA content increased from 42 to 49 mol%. These results suggest that the drastic change in enzymatic degradability for these copolymer films is caused not only by their chemical structure but also by the affinity of lipase for the surface of these copolymer films and by the morphology such as the crystal structure and the higher-order structure. The T_m of these copolymers is also influenced by the morphology. The distinct structure of the used lipase from *Rhizopus arrhizus* has not been reported. However, many researchers[37,50-52] have reported that the activity of a lipase is exhibited for the substrate after

Table II. Crystalline reflections for two representative copolymer films

Copolymer		2θ-Position			
Polym. comp. SA/EO/mol%	M_n				
42/58	47000	20.2	22.8	23.3	27.0
48/52	34700	20.2	22.9	23.4	26.9

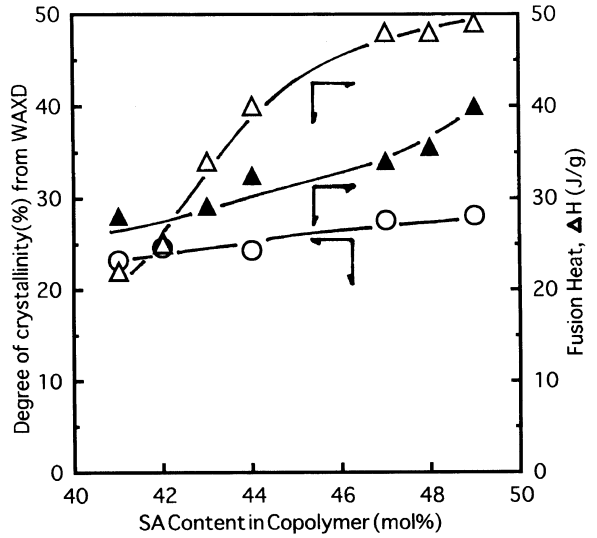

Figure 5. Degree of crystallinity (○), fusion heat (△) of the original copolymers, or fusion heat (▲) of the copolymer films *versus* SA content in the extended copoly(SA/EO) films.

the adsorption of the enzyme on the substrate surface. Therefore, the ease of the adsorption of the lipase on the surface of the polymer film will affect the enzymatic activity.

In order to evaluate the ease of the adsorption of the lipase on the surface of the polymer films, the water absorption of these copolymer films and the contact angle of distilled water on the surface of these copolymer films were measured. The obtained data are shown in Figure 4. The change of the water absorption compared to one of the SA content were almost within the margin of the experimental error. The contact angles were about 60° and only slightly increased with an increase in SA content. These data suggest that the hydrophobic character of these copolymer films negligibly increases with the increase in SA content. From the water adsorption and the contact angle results, it is deduced that the adsorption probabilities of the lipase on the surface of the copolymer films are kept, rather surprisingly, constant independent of any variation in SA content in these copolymer films.

The next step was the evaluation of these copolymer films morphology by DSC, WAXD and SAXS. The WAXD patterns of all copolymer films, independently of SA content, did not show any appreciable variation. The locations of crystalline reflections of some typical copolymer films are summarized in Table II. Figure 5 shows the degree of crystallinity from WAXD, calculated according to Ruland's method,[49] and the fusion heat of crystal from the DSC endotherm peaks of these copolymers. The degree of crystallinity slightly rose from 24 to 28% as the SA content changes from 42 to 49 mol%.

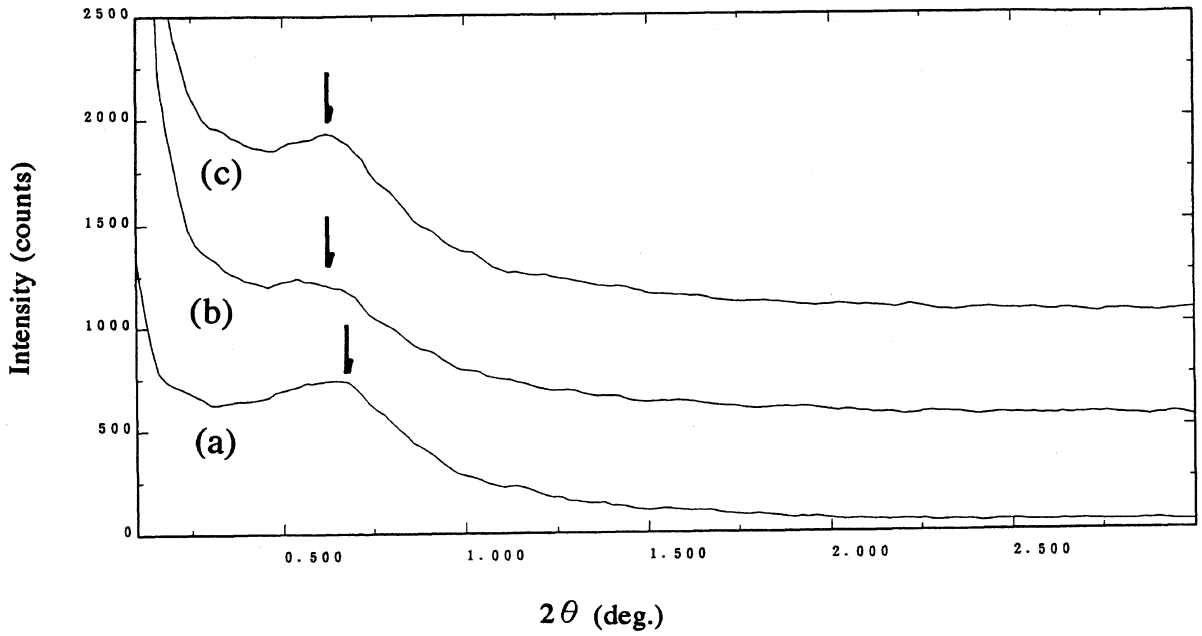

Figure 6. SAXS intensity distribution for the extended copoly(SA/EO) films (M_n=48700, polym. comp., SA/EO=41/59) after enzymatic hydrolysis by the lipase (50000U) from *Rhizopus arrhizus*. (a) before enzymatic hydrolysis test; (b) 30 wt% loss; (c) 60 wt% loss.

Fusion heat (ΔH) of the original copolymers after precipitation from petroleum ether, determined from DSC (first scan), showed an increase as SA content increased. The ΔH changes of these copolymer *versus* the SA content were smaller than one of the original copolymers. In general, the T_m is given by equation 2: where ΔH_m is the enthalpy of

$$T_m = \Delta H_m / \Delta S_m \quad (2)$$

fusion, ΔS_m is the entropy of fusion. The ΔH is nearly identical with the ΔH_m under the assumptions that other transformation, such as recrystallization and glass transition, except for the crystal fusion did not occur. We previously reported that the T_m and the glass transition temperature (T_g) of these copolymer films were identical with those of the original copolymers and rose with an increase in the SA content of these copolymers.[48] Since the ΔH values of these copolymer films were smaller than those of the original copolymers, despite the T_m rising with the increase of SA content, it is apparent that the ΔS_m of their films are smaller than the original copolymers and their ΔS_m decreases with an increase in SA content. These results indicate that the arrangement of molecular chains in our copolymer films become by far more disordered than their original copolymers as SA content decreases. The previous experimental results showed that the T_g of these copolymers rose from -20 to -10°C as the SA content increased from 42 to 49 mol% thus confirming the assumption that the molecular motions of polymer chain in amorphous regions are further restricted by an increase in SA content.

SAXS measurements were taken in an attempt to clarify the higher-order structure in these copolymer films. Figure 6a shows the result of SAXS intensity distribution of the copolymer film (SA/EO=42/58, M_n=37000). A primary scattering peak was observed, though its scattering strength was small but no second and third peak were observed. Similarly, their profile of SAXS intensity distribution and their peak strength were obtained for other copolymer films. Following these results, our copolymer films have an alternating crystalline layer structure including considerable lattice defects. The long-periods between crystal lamellae were calculated from the scattering angles of the primary scattering peaks. The change of the long-period with an increase in SA content of the copolymer films is shown in Figure 7. The long-period decreased from 110 to 80 Å as SA content increased from 42 to 49 mol%. In general, multiplication of the long-period by the degree of crystallinity gives the thickness of lamelae. The amorphous region size is approximated by eq 3.

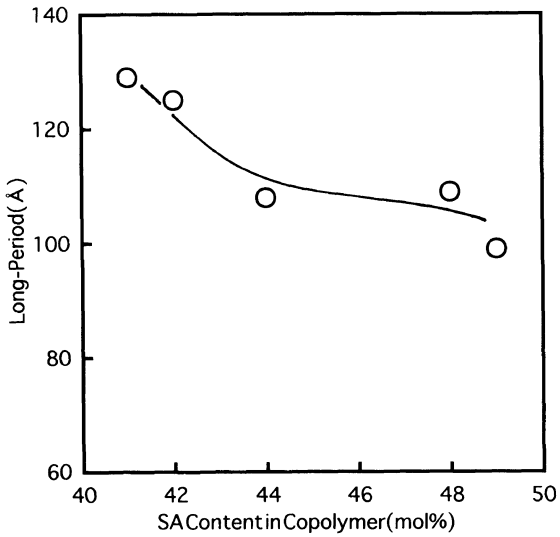

Figure 7. Long-period of the lamellar structure against SA content in the extended copoly(SA/EO) films.

Table III. Degree of crystallinity and long-period of extended copoly(SA/EO) film[a] before and after enzymatic hydrolysis[b]

Erosion Time	Weight Loss	Degree of crystallinity[c]	Long-period[d]
h	%	%	Å
0	0	23.2	129.8±2
18	30	22.6	133.7±2
48	60	22.8	133.7±3

[a] Film was prepared by compression molding. The M_n and the composition, (SA/EO), of copoly(SA/EO) was 48700 and 41/59, respectively. [b] Lipase from *Rhizopus arrhizus* (5000U) was used. Temprerature, 37°C; pH, 7.0. [c] By wide-angle X-ray diffraction. [d] By small-angle X-ray scattering (three times measurments).

$$\text{Amorphous region size} = \text{Long period} - \text{Thickness of lamellae} \quad (3)$$

From the WAXD results, the change in the thickness of lamelae *versus* SA content for the copolymer films was found to be small. Consequently, the change of long-period can be identified as one of the parameters determining the amorphous region size. The SAXS profiles of these copolymer films after enzymatic hydrolysis are shown in Figure 6. Table III shows the change of the long-period and the degree of crystallinity of the copolymer films after enzymatic hydrolysis. The degree of crystallinity very slightly decreased when weight loss of the copolymer films was 30 wt% or even 60 wt%. At the same time, the SAXS of films, before and after enzymatic hydrolysis, did not show any substantial change. A certain increase (4 Å) in long-period after enzymatic hydrolysis was observed from the SAXS results. These results suggest that small change in the crystal lamellar region occurred and the amorphous region and the intermediate region between the crystal lamellar region and the amorphous region were enzymatically degraded. Furthermore, the amorphous region more likely expanded by enzymatic hydrolysis.

The enzymatic degradation behavior, exhibited by the extended copoly(SA/EO) films in the presence of the lipase from *Rhizopus arrhizus*, could be summarized as follows: The extended copoly(SA/EO) films have an alternating crystalline layer structure due to their film processing. The molecular motion of a polymer chain in the amorphous phase is limited by the crystalline phase neighboring at both sides of the amorphous phase. Generally, the T_g temperature of a polymer shows the extent of molecular motion in amorphous region. In the case of the lamellar model, the T_g is considered to involve defect regions within or at the boundaries of the lamellae. It was reported above that the T_g of the copoly(SA/EO) films rose from -20 to -10°C as SA content increased. Since the long-period decreased and the T_g of the copolymer films shifted to a higher temperature with the increase in SA content, it becomes obvious that the molecular motion of a polymer chain in the amorphous region drops as SA content increases from 42 to 49 mol%. Furthermore, the probabilities of adsorption of the lipase (from *Rhizopus arrhizus*) on the copolymer films were not at all affected by any variation of SA content. Many researchers previously reported that the sizes of many kinds of lipase are ca 100 Å.[53-56] Therefore, by taking into account the size of an enzyme, the distance of the amorphous region and the extent of the molecular motion in the amorphous region can be predicted in order to optimize the enzymatic degradation of the copolymer films which have an alternating crystalline structure.

CONCLUSIONS

The morphology and the enzymatic degradation behavior of the extended copoly(SA/EO) films by the lipase from *Rhizopus arrhizus* were investigated and our finding can be summarized as follows:

(1) The extended copoly(SA/EO) film had the same crystalline structure irrespectively of any change in SA content. The degree of crystallinity was within a range from 24 to 28% as SA content increased from 42 to 49 mol%.

(2) The extended copoly(SA/EO) films were composed of an alternating crystalline layer structure and the long-period between lamellar crystalline increased from 80 to 110 Å with a decrease in SA content.

(3) The enzymatic degradability of our copolymer films by lipase was shown to decrease considerably with an increase in SA content of these copolymers.

(4) The higher-order structure had a great impact on the enzymatic degradation behavior.

Acknowledgement. The authors are grateful to Mr. Tobita of Rigaku Corporation for his suggestions on X-ray measurement.

REFERENCES

1. W. Schnabel, "Polymer Degradation; Principles & Applications," Hanser International, Munich, 1981, pp 154—176.
2. S. Kumar, "Biodegradable Polymers," Marcel Dekker Co., New York, N.Y., 1987, pp 4—72.
3. D. Satyranayana and P. R. Chaterji, *J. Macromol. Sci., Rev. Macromol. Chem. Phys.*, **C33**, 349 (1993).
4. I. Aranitoyannis, A. Nakayama, N. Kawasaki, and N. Yamamoto, *Angew. Makromol. Chem.*, **222**, 111 (1994).
5. I. Aranitoyannis, A. Nakayama, N. Kawasaki, and N. Yamamoto, *Polymer*, **35**, 1380 (1994).
6. I. Aranitoyannis, A. Nakayama, N. Kawasaki, and N. Yamamoto, *Polymer*, **36**, 857 (1995).
7. I. Aranitoyannis, E. Nikolaou, and N. Yamamoto, *Makromol. Chem.*, **196**, 1129 (1995).
8. I. Aranitoyannis, E. Psomiadou, N. Yamamoto, and J. M. V. Blanshard, *J. Appl. Polym. Sci.*, **56**, 1045 (1994).
9. A. L. Andrady, *J. Macromol. Sci., Rev. Macromol. Chem. Phys.*, **C34**(1), 25 (1995).
10. E. Chiellini and R. Solaro, *Chem. Technol.*, **23**, 29 (1993).
11. K. Yamada, K. Mukai, and Y. Doi, *Int. J. Biol. Macromol.*, **15**, 215 (1993).
12. R. H. Marchssault, S. Coulombe, H. Morikawa, K. Okumura, and J. F. Revol, *Can. J. Chem.*, **59**, 38 (1981).
13. J. Mergaert, A. Webb, C. Anderson, A. Wouters, and J. Swings, *J. Appl. Environ. Microbiol.*, **59**, 3233 (1993).
14. D. Jendrossek, I. Knobe, R. B. Habibian, A. Steibuchel, and H. G. Schlegel, *J. Environ. Polym. Degrad.*, **1**, 53 (1993).
15. P. P. King, *J. Chem. Tec. Biotecnol*, **32**, 2 (1982).
16. Y. Doi, M. Kunioka, Y. Nakamura, and K. Soga, *Macromolecules*, **21**, 2722 (1988).
17. E. Takiyama and T. Fujimaki, "Biodegradable Plastics and Polymers," Vol. 12, Y. Doi and K. Fukuda, Ed., Elsevier, Amsterdam, 1994, Session 3, pp 150—174.
18. P. Dubois, C. Jacobs, R. Jerome, and Ph. Teyssie, *Macromolecules*, **24**, 2266 (1991).

19. Y. Kimura and K. Shirotani, *Macromolecules*, **21**, 3338 (1988).
20. Y. Tokiwa and T. Suzuki, *Nature*, **270**, 76 (1977).
21. T. Yasuda, T. Aida, and S. Inoue, *Macromolecules*, **17**, 1089 (1981).
22. H. R. Kricheldorf, T. Mang, and M. Jonte, *Macromolecules*, **17**, 2173 (1984).
23. Y. Zhang, R. A. Gross, and R. W. Lenz, *Macromolecules*, **23**, 3206 (1990).
24. A. Nakayama, N. Kawasaki, I. Arvanitoyannis, J. Iyoda, and N. Yamamoto, *Polymer*, **36**, 1295 (1995).
25. H. Fukuzaki, M. Yoshida, M. Asano, Y. Aiba, and I. Kaetsu, *Eur. Polym. J.*, **24**, 1029 (1988).
26. A. M. Reed and D. K. Gilding, *Polymer*, **22**, 494 (1981).
27. J. Xu, R. A. Gross, D. L. Kaplan, and G. Swift, *Macromolecules*, **29**, 3857 (1996).
28. T. Ouchi and A. Fujino, *Makromol. Chem.*, **190**, 1523 (1989).
29. A-C. Albertsson and A. Lofgren, *J. Macromol. Sci.*, **A32**, 41 (1995).
30. I. Arvanitoyannis, A. Nakayama, E. Psomiadou, N. Kawasaki, and N. Yamamoto, *Polymer*, **37**, 651 (1996).
31. I. Arvanitoyannis, A. Nakayama, N. Kawasaki, and N. Yamamoto, *Polymer*, **36**, 2271 (1995).
32. I. Arvanitoyannis, A. Nakayama, N. Kawasaki, and N. Yamamoto, *Polymer*, **36**, 2947 (1995).
33. Y. Yamashita, Y. Taketomoto, E. Ihara, and H. Yasuda, *Macromolecules*, **29**, 1798 (1996).
34. Y. Maeda, A. Nakayama, N. Kawasaki, K. Hayashi, S. Aiba, and N. Yamamoto, *Polymer*, in press.
35. F. Kawai, *Kobunshi Ronbunshu*, **50**, 775 (1993).
36. F. Kawai, *Polym. Mater. Sci. Eng.*, **62**, 979 (1990).
37. T. Walter, J. Augusta, R. J. Muller, H. Widdecke, and J. Kelein, *Enzyme Microb., Technol.*, **17**, 218 (1995).
38. S. J. Holland, A. M. Jolly, M. Yasin, and B. J. Tighe, *Biomaterials*, **8**, 289 (1987).
39. M. Kunioka, A. Tamaki, and Y. Doi, *Macromolecules*, **23**, 694 (1989).
40. Y. Doi, Y. Kanesawa, and M. Kunioka, *Macromolecules*, **23**, 26 (1990).
41. H. Nishida and Y. Tokiwa, *J. Environ. Polym. Degrad.*, **1**, 65 (1993).
42. K. Mukai, K. Yamada, and Y. Doi, *Int. J. Biol. Macromol.*, **15**, 361 (1993).
43. H. Abe, I. Matsubara, and Y. Doi, *Macromolecules*, **28**, 844 (1995).
44. P. J. Hocking, M. R. Timmins, T. M. Scherer, R. C. Fuller, R. W. Lenz, and R. H. Maechessault, *Macromol. Rapid Commun.*, **15**, 447 (1994).
45. J. J. Jesudason, R. H. Marchessau, and T. Saito, *J. Eviron. Polym. Degrad.*, **1**, 89 (1993).
46. Y. Kumagai, Y. Kanesawa, and Y. Doi, *Makromol, Chem.*, **193**, 53 (1992).
47. Y. Maeda, A. Nakayama, J. Iyoda, K. Hayashi, and N. Yamamoto, *Kobunshi Ronbunshu*, **50**, 723 (1993).
48. Y. Maeda, A. Nakayama, N. Kawasaki, K. Hayashi, S. Aiba, and N. Yamamoto, *J. Environ. Polym. Degrad.*, **4**, 225 (1996).
49. W. Ruland, *Polymer*, **5**, 89 (1964).
50. Y. Tsujisaka, M. Iwai, S. Okumura, Y. Tominaga, A. Sugihara, Y. Shimada, *Bull. Osaka Municipal Technical Research Institute*, **62**, 72 (1981).
51. F. H. Mattson, R. A. Volpenhein, and L. Benjamin, *J. Biol. Chem.*, **245**, 5535 (1970).
52. Y. Gargouri, G. Pieroni, C. Riviere, A. Sugihara, L. Sarda, and R. Verger, *J. Biol. Chem.*, **260**, 2268 (1985).
53. M. Semeriva, G. Benzonana, and P. Desnuelle, *Biochim. Biophys. Acta.*, **191**, 598 (1969).
54. A. Park and L. G. Cima, *J .Biomed. Mater. Res.*, **31**, 117 (1996).
55. Y. Hata, Y. Matsuura, N. Tanaka, M. Kakudo, A. Sugihara, and M. Iwai, *J. Biochem.*, **86**, 1821 (1979).
56. A. Sugihara, Y. Tominaga, T. Iizumi, and S. Tsunasawa, *J. Biochem.*, **106**, 383 (1989).