

Hydrolysis of Copolyesters Containing Aromatic and Aliphatic Ester Blocks by Lipase

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Synopsis

Copolyesters (CPEs) prepared by the transesterification reaction between aromatic and aliphatic polyesters were hydrolyzed by *Rhizopus delemar* lipase. The susceptibility of CPEs to hydrolysis by this lipase dropped off rapidly during the initial stage of the transesterification reaction and increased gradually as the reaction proceeded. The susceptibility to hydrolysis decreased with increase in aromatic polyester content. It was concluded that the rigidity of the aromatic ring in the CPE chains strongly influenced their susceptibility to hydrolysis by this lipase.

INTRODUCTION

It is generally known that among synthetic condensation polymers polyamides (nylons) and aromatic polyesters are nonbiodegradable although aliphatic polyesters are susceptible to biologic attack.¹⁻³ We reported previously that the amount and distribution of the hydrogen bonds, based on the amide group, in the copolyamide esters strongly influenced their susceptibility to hydrolysis by *Rhizopus delemar* lipase.⁴ So it is assumed that the hydrogen bonds in the polyamide chains are one of the factors which render polyamides resistant against biologic attack. In order to find the reason why aromatic polyesters are not biodegradable, we prepared various copolyesters (CPEs) by the transesterification reaction between aromatic and aliphatic polyester and then studied their susceptibility to *Rh. delemar* lipase, which is capable of hydrolyzing aliphatic polyesters.

EXPERIMENTAL

Materials

Polycaprolactone (PCL; \bar{M}_n 25,000) and poly(butylene terephthalate) (PBT) chips were purchased from Union Carbide Corp. Poly(ethylene terephthalate) (PET) chips were kindly supplied by Asahikasei Kogyo Co., Ltd. Poly(ethylene isophthalate) (PEIP) was purchased from Nihon Chromato Works, Ltd. Copolyester of PET and poly(cyclohexylenedimethyl succinate) (PETG) were purchased from Eastman Chemical Products, Inc. Anhydrous zinc acetate was prepared by maintaining zinc acetate hydrate at 140°C for 4 hr. Ultracentrifugally homogeneous preparation of *Rhizopus delemar* lipase was purchased from Seikagaku Kogyo Co., Ltd. This lipase randomly splits the ester bonds of aliphatic polyester, finally degrading it to the constituent units.

Preparation of Copolyesters

CPEs were synthesized by the transesterification reaction between aromatic polyester and aliphatic polyester.⁵⁻⁷ Aliphatic polyester was added to molten aromatic polyester at about 270°C in a nitrogen atmosphere with 0.5 wt % of anhydrous zinc acetate, and the mixture was stirred. As in the time course of a typical transesterification reaction, it was found that CPEs with large blocks were formed in the first stage, the blocks then became shorter, and finally, at equilibrium, random copolymers were formed.

The progress of the transesterification reaction can be followed by differential scanning calorimetry (DSC). As the reaction proceeded, the position of the fusion peaks on the high-temperature side, which revealed the reaction between PCL and PBT (molar ratio 50:50), were plotted (Fig. 1) as a function of the transesterification reaction time. The progress of the transesterification reaction was also ascertained by the change in the solubility of the CPEs in chloroform.

Furthermore, the formation of CPEs was confirmed by infrared analysis after the reaction products were fractionated with chloroform, a good solvent of PCL. A mixture of PCL and PBT (molar ratio, 50:50) that was blended at 270°C for 10 min without catalyst, although this meant that a slight transesterification reaction might occur, underwent almost complete separation into PCL and PBT after chloroform extraction. On the other hand, when a mixture of PCL and PBT (molar ratio 50:50) was submitted to the transesterification reaction for 2 hr with a catalyst, similar absorption patterns were obtained in both the chloroform-insoluble part and the chloroform-soluble fraction. The molecular weight distribution of the CPEs, which was estimated by gel permeation chromatography (GPC) using model HLC-802R instruments (Toyo Soda Industry Co., Ltd.), did not change significantly during the course of the transesterification reaction.

Assay of Biodegradability (Susceptibility to Hydrolysis by Lipase) of CPEs

The biodegradability of the CPEs was assayed by the rate of their solubilization when lipase acted on them. This enzyme assay system does not necessarily in-

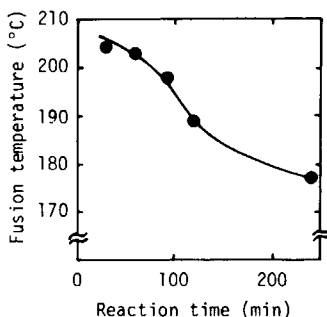


Fig. 1. Fusion temperature depression for PCL-PBT (molar ratio 50:50) system as function of the transesterification reaction time. DSC was carried out using a Rigakudenki DTA 8001 apparatus equipped with a DSC sample holder. Conditions of DSC were as follows: sample, 2-4 mg; heating rate, 5°C/min; range, ± 1 mcal/sec; air atmosphere.

volve complete degradation into the constituent units. The reaction mixture contained 100 μ mole phosphate buffer (pH 7.0), 0.1 mg Plysurf A210G, CPE powder or its films (20 mg as aliphatic polyester moiety), and 0.2 mg *Rhizopus delemar* lipase in a total volume of 1.0 ml. Reaction mixtures were incubated on a shaker at 150 rpm at 37°C for 16 hr. After incubation, the water-soluble total organic carbon (TOC) concentration in the filtrate of the reaction mixture was measured with a Beckman TOC analyzer. Formation of the water-soluble TOC was in proportion to substrate amounts (up to 50 mg as aliphatic polyester moiety) in this reaction system. The CPEs were powdered by grinding or were cut into film (0.20–0.27 mm thick). The biodegradability of the CPEs was expressed by assuming that the water-soluble TOC in the reaction mixture was formed only because of the aliphatic polyester blocks.

RESULTS

Effect of Transesterification Reaction Time on Biodegradability of CPEs

The biodegradability fell off remarkably during the initial stage of the transesterification reaction, when, presumably, the CPEs had relatively large blocks. But as the reaction proceeded, that is, with shortening of the CPE blocks, it increased gradually as shown in Figures 2 and 3.

In order to confirm that the TOC formation in Figures 2 and 3 was really based on CPE and not on unreacted PCL, CPEs composed of PET and PCL (molar ratio 10:90; reaction time for synthesis 13 min) were extracted with benzene to remove unreacted PCL. CPE of the benzene-insoluble fraction (33% yield) contained an abundance of PET blocks, but no unreacted PCL. Using 23.8 mg of this fraction, the biodegradability was examined in a similar manner. Water-soluble TOC corresponding to 41% of that of CPE before extraction with benzene was formed. Thus, it was confirmed that CPE can be hydrolyzed by lipase.

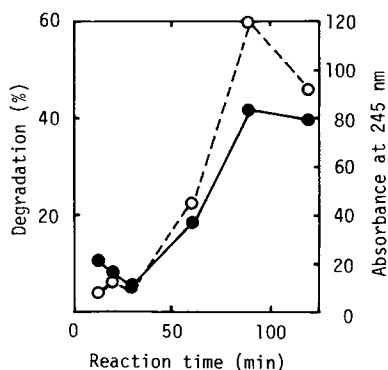


Fig. 2. Effect of transesterification reaction time on the biodegradability of CPE composed of PCL–PET (molar ratio 90:10) system. Solid and broken lines indicate degradation of CPE and absorbance at 245 nm (absorbance 10 corresponds to 11 ppm terephthalate) of the soluble fraction in the reaction mixture, respectively. When PCL alone was used as the substrate in this reaction system, the degradation was about 90%.

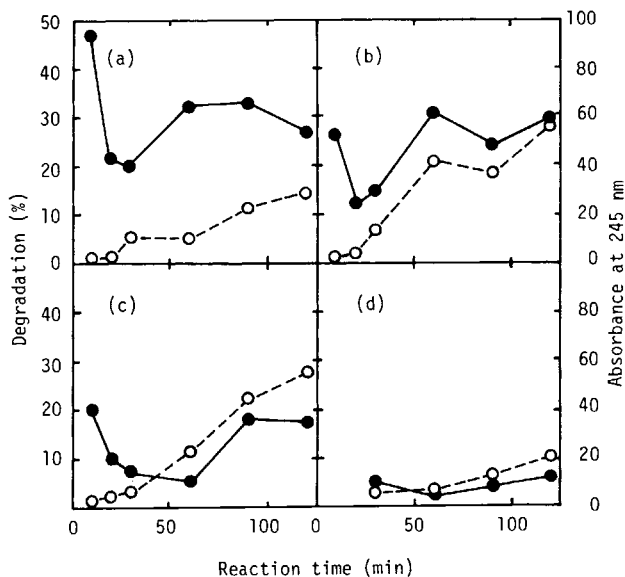


Fig. 3. Effect of transesterification reaction time on the biodegradability of CPE composed of PCL-PBT system. Molar ratios of PCL and PBT are: (a) 95:5; (b) 90:10; (c) 80:20, and (d) 50:50. Significance of solid and broken lines is the same as in Fig. 2.

Figure 4 shows the biodegradability of CPEs composed of PCL and PEIP, the latter being used as a low- T_m (103°C) aromatic polyester. These CPEs were synthesized at 170°C because CPEs synthesized at 270°C had low molecular weights and T_m values which were too low to permit examination of their biodegradabilities in this reaction system. The biodegradabilities of these CPEs were fairly high compared to those of other CPEs, although they declined slightly during the first stage of the transesterification reaction.

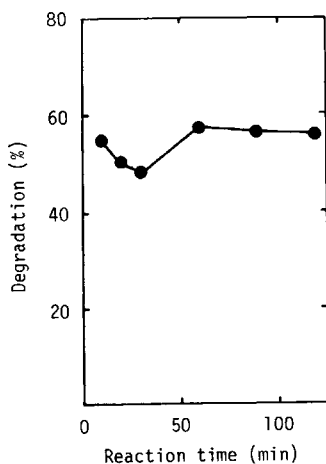


Fig. 4. Effect of transesterification reaction time on the biodegradability of CPE composed of PCL-PEIP (molar ratio 90:10) system.

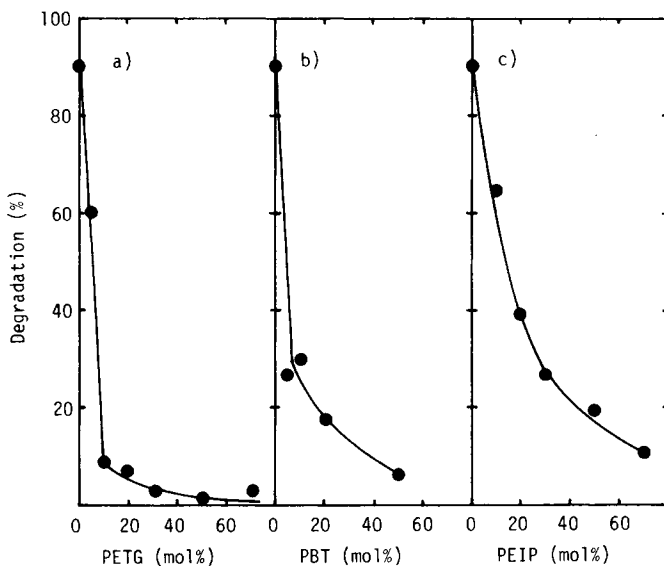


Fig. 5. Effect of molar ratio of PCL and aromatic polyester on the biodegradability of CPE by *Rhizopus delemar* lipase; (a), (b), and (c) indicate PCL-PETG, PCL-PBT, and PCL-PEIP systems, respectively. The reaction conditions for each CPE synthesis were (a) 270°C for 2 hr, (b) 270°C for 2 hr, and (c) 170°C for 2 hr.

Effect of Molar Ratio of Aliphatic Polyester and Aromatic Polyester on Biodegradability

As shown in Figure 5, the biodegradability of CPEs decreased with increase in aromatic polyester content and also depended on the compositions of the CPEs. The biodegradabilities of CPEs composed of PCL and PEIP were greater than those of other CPEs.

Effect of Chemical Structure of CPEs on Biodegradability

Figure 6 shows the biodegradabilities of various CPEs.

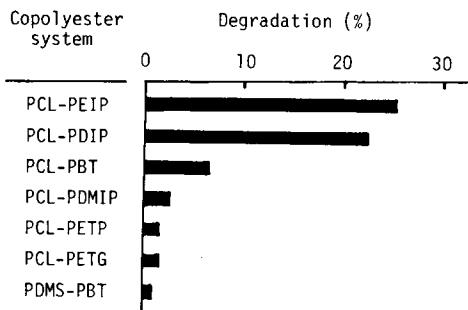


Fig. 6. Degradabilities of various CPEs by *Rh. delemar* lipase. The molar ratio of each CPE of aliphatic polyester and aromatic polyester was 50:50, and reaction time for each CPE synthesis was 2 hr. PDMIP: poly(2,2-dimethyltrimethylene isophthalate); PETP: poly(ethylene tetrachlorophthalate); PDMS: poly(2,2-dimethyltrimethylene succinate).

The susceptibilities to hydrolysis by lipase of CPEs containing PEIP or poly(diethylene isophthalate) (PDIP), which showed relatively low T_m , were high, but those of CPEs composed of polyesters which contained side chains or chlorine were low in spite of low- T_m polyester.

Degradation Products of CPE by Lipase

The degradation products contained a substance which has an absorption maximum near $245 \mu\text{m}$ as shown in Figures 2 and 3. The gas-liquid chromatograms of the degradation products after esterification with diazomethane are shown in Figure 7. Terephthalic acid was detected in addition to ϵ -hydroxycaproic acid and its oligomers based on PCL.

DISCUSSION

The majority of low-molecular-weight artificial synthetic compounds are biodegradable, whereas only few polymers are biodegradable. Concerning water-insoluble condensation polymers, we noted that the T_m had a great effect on biodegradability. Figure 8 shows the relationship between the T_m and the biodegradability of saturated aliphatic polyesters. For the same series polyesters, the biodegradabilities decreased with increasing T_m . Unfortunately, the softening points (T_m) of aliphatic polyesters are too low to permit their use in many fields. On the other hand, the T_m values of aliphatic polyamide (nylon) and aromatic polyester are high enough to permit their use in various fields although

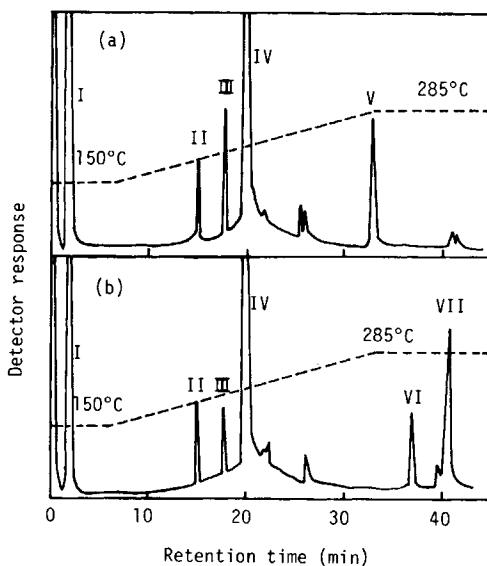


Fig. 7. Gas chromatograms of the degradation product of CPE composed of (a) PCL-PET and (b) PCL-PBT (molar ratio 90:10; transesterification reaction time 2 hr) by *Rh. delemar* lipase. Chromatographic conditions: Shimadzu GC-5A (flame ionisation) with glass column ($2 \text{ m} \times 0.3 \text{ cm}$ internal diameter) packed with Silicon OV-17 on 60/80 mesh Unipor B. Temperature programmed at $5^\circ\text{C}/\text{min}$ from 150 to 285°C (dashed line). Flow rate of both nitrogen and hydrogen was $50 \text{ ml}/\text{min}$: I, methyl ester of ϵ -hydroxycaproic acid; II, dimethyl terephthalate; III and IV, degradation products or methyl ester thereof based on PCL; V, VI, and VII, unknown products.

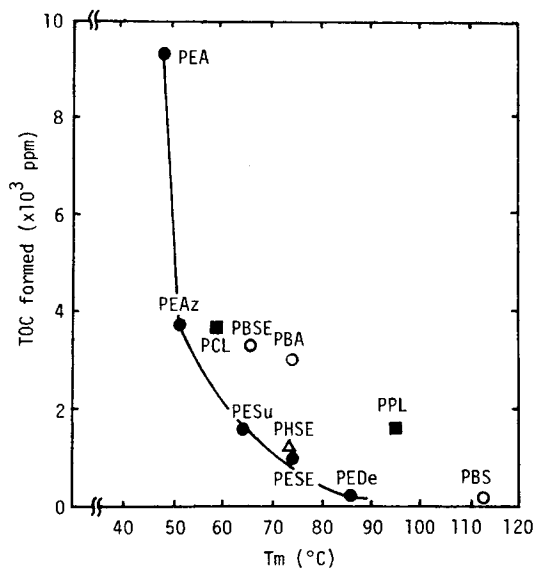


Fig. 8. Relationship between melting point (T_m) and the biodegradability of saturated aliphatic polyesters by *Rh. arrhizus* lipase. Biodegradability was examined according to the method previously reported,³ except that *Rh. arrhizus* lipase was used instead of *Rh. delemar* lipase. T_m of each polyester was measured by Yanaco micro melting point apparatus (model MP-S3). PEA: poly(ethylene adipate); PEAz: poly(ethylene azelate); PESu: poly(ethylene suberate); PESE: poly(ethylene sebacate); PEde: poly(ethylene decamethylate); PBS: poly(butylene succinate); PBA: poly(butylene adipate); PBSE: poly(butylene sebacate); PHSE: poly(hexamethylene sebacate); PPL: polypropiolactone.

they are not biodegradable. In general, T_m was represented by the following formula:

$$T_m = \Delta H / \Delta S$$

where ΔH is the change of enthalpy in melting and ΔS is the change of entropy in melting.

It is known that the interactions among polymer chains mainly affect the ΔH value and that the internal rotation energies corresponding to the rigidity (the flexibility) of the polymer molecule remarkably affect the ΔS value. The high T_m of nylon is caused by the large ΔH value based on the hydrogen bonds among polymer chains; on the contrary, the high T_m of aromatic polyester is caused by the small ΔS value with increase in the rigidity (decrease in flexibility) of the polymer molecule based on an aromatic ring (Fig. 9). We reported previously that the ΔH value in the copolyamide ester had a strong effect on biodegradation.⁴

In order to find the effect of the ΔS value on biodegradability, we synthesized CPEs by the transesterification reaction between aliphatic polyesters and aromatic polyesters and then studied their susceptibility to hydrolysis by *Rh. delemar* lipase. This lipase randomly splits the ester bonds of aliphatic polyester, finally degrading it into its constituent units.

The susceptibility to hydrolysis by the lipase was remarkably low in the initial stage of the transesterification reaction (Figs. 2 and 3) and decreased with increasing aromatic polyester content (Fig. 5). It was concluded that the susceptibility to hydrolysis of CPEs by the lipase decreased with increasing rigidity

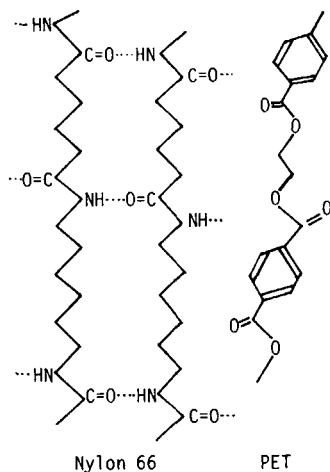


Fig. 9. Structure of nylon 66 [poly(hexamethylene adipamide)] and PET.

of the CPE molecule based on the aromatic ring. However, when the transesterification reaction proceeded sufficiently and the T_m decreased correspondingly to the decrease in the rigidity of the CPE molecule, the susceptibility to hydrolysis increased (Figs. 2 and 3). CPEs containing PEIP or PDIP as low- T_m aromatic polyester were hydrolyzed better than other CPEs (Fig. 6). Hence, it was found that the rigidity of CPE molecular chains, which is related to the ΔS value, had a great effect, in addition to the chemical structure, on biodegradability.

As the degradation products of CPEs composed of PCL and PBT or PET, terephthalic acid was detected in addition to ϵ -hydroxycaproic acid and its oligomers based on PCL.

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