Synthesis of Copolyamide–Esters and Some Aspects Involved in Their Hydrolysis by Lipase

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Synopsis

Copolyamide–esters (CPAEs) were synthesized by the amide–ester interchange reaction. The change of intrinsic viscosity during CPAE synthesis was negligible. Polyamide blocks were shortened with increasing reaction time and polyester content. The polymerization degree of nylon 12 blocks on CPAE was smaller than that of nylon 6 blocks. CPAEs were hydrolyzed by *Rhizopus delemar* lipase. The biodegradability decreased with the shortening of the polyamide blocks and with increasing polyamide content. It was concluded that the amount and distribution of the hydrogen bonds, based on the amide group, in the CPAE chains strongly influenced their biodegradation by this lipase.

INTRODUCTION

We reported previously that synthetic aliphatic polyesters were hydrolyzed by several commercial lipases and hog liver esterase.[1] Unfortunately, the softening points (T_m) of synthetic aliphatic polyesters are too low to permit their use as a material in various fields. On the other hand, all forms of aliphatic polyamides (nylons), except for their oligomers,[2–4] are known to be nonbiodegradable. In order to find both new biodegradable synthetic polymers and the reason why aliphatic polyamides are not biodegradable, we synthesized copolyamide–esters (CPAEs) by the amide–ester interchange reaction between polyamide and polyester and then studied their susceptibility to hydrolysis by lipase.

MATERIALS AND METHODS

Materials. Polycaprolactone (PCL; $\overline{M}_n$ 25,000) chips were purchased from Union Carbide Corp. Nylon 6 (polycaprolactam; $[\eta]$ = 1.30 dl/g in m-cresol at 25°C) chips were kindly supplied by Toyokasei Kogyo Co., Ltd. Nylon 66 (polyhexamethylene adipamide), nylon 69 (polyhexamethylene azelamide), and nylon 612 (polyhexamethylene dodecandiamide) chips were purchased from Aldrich Chemical Co., Inc. Nylon 11 [poly(11-aminoundecanoic acid)] and nylon 12 [poly(12-aminolauric acid)] chips were kindly supplied by Nihon Lilsan Corp. Anhydrous zinc acetate was prepared by maintaining zinc acetate hydrate at 140°C for 4 hr. The ultracentrifuged homogeneous preparation of *Rhizopus delemar* lipase was purchased from Seikagaku Kogyo Co., Ltd.

Synthesis of CPAE. CPAEs were synthesized by the amide–ester interchange reaction between polyamide and polyester.[5] Mixtures of polyamide and polyester were heated to about 270°C in a nitrogen atmosphere with 0.5% of

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anhydrous zinc acetate and were stirred when the mixtures began to melt after a few minutes. As in the time course of a typical amide–ester interchange reaction, it was found that CPAEs with large blocks were formed in the first stage, the blocks were then shortened, and in the final stage random copolymers were formed.

Preparation of Polyamide and Polyester Blends. Blends of polyamide and polyester were prepared by stirring the mixture of polyamide and polyester at 270°C for 10 min without catalyst, although this meant that a slight amide–ester interchange reaction might occur.

Differential Scanning Calorimetry of Polymers. DSC was carried out by 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.

Biodegradability Assay of CPAEs. The biodegradability of the CPAEs was assayed by the rate of their solubilization when lipase acted on them. This enzyme assay system does not necessarily involve complete degradation into the constituent units. The reaction mixture contained 100 μmol of phosphate buffer (pH 7.0), 0.1 mg of plysurf A210G, CPAE powder or its films (20 mg as polyester moiety), and 0.2 mg of *Rh. 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 polyester moiety) in this reaction system. The CPAEs were powdered by grinding or were cut into film (0.20–0.27 mm thick). The biodegradability of the CPAEs was represented by assuming that the water-soluble TOC in the reaction mixture was formed only due to the polyester blocks.

Hydrolysis of Ester Bonds in CPAEs. Ester bonds in the CPAEs were hydrolyzed by alcoholic alkali. Two g of CPAE was added to a mixture solution containing 10 g of water, 2.5 g of potassium hydroxide, and 37.5 g of ethyl alcohol; the solution was then hydrolyzed at 30°C for 3 or 4 days. The pH of the solution after removal of the alcohol was adjusted to 2, and the solution was then filtrated through a glass filter. The precipitate obtained was dried *in vacuo* and was used as polyamide blocks from CPAE.

Determination of Carboxylic Group Terminals. The carboxylic group terminals were determined by alkali titration.[6] After 200 mg of the sample was dissolved in 7.5 ml of benzyl alcohol in a nitrogen atmosphere at 110°C, the solution was titrated with 0.05*N* potassium hydroxide using phenolphthalein as an indicator. A blank test was carried out by the same method but omitting the sample.

Estimation of Molecular Weight Distribution of Polyamide Blocks. The molecular weight distribution of the polyamide blocks was estimated by gel-permeation chromatography (GPC) using two instruments, model HLC-802R (Toyo Soda Industry Co., Ltd.) and model GPC-244 (Waters Associates, Inc.). The conditions for the first instrument were as follows: sample, 0.75 mg; columns, $GMH_4 \times 2$; column temperature, 40°C; solvent, m-cresol/chloroform (1/4) containing 0.5% benzoic acid; pressure, 30 kg/cm^2; flow rate, 1.3 ml/min; detector, differential refractometer. The conditions for the second were as follows: sample, 2.5 mg; columns, GMH_6 and $G2000H_8$; column temperature, room

temperature; solvent, m-cresol/chloroform (1/4); pressure, 42 kg/cm^2; flow rate, 1.0 ml/min; detector, differential refractometer. Polystyrene and nylon olgiomer were used as standards.

RESULTS

Formation of CPAE

The processes of the amide–ester interchange reaction can be followed by DSC. Fusion peaks in heating, which revealed the reaction between nylon 12 and PCL (molar ratio, 37:63), were as shown in Figure 1. As the reaction proceeds, the position of the fusion peaks shifted to lower temperatures, and their shape broadened gradually, possibly indicating a decrease in crystallinity. When the reaction times were 120 and 240 min, the fusion peak due to PCL on the low-temperature side was not detected. Similar changes in DSC thermograms were observed in the reaction of PCL and another nylon. The temperatures of the fusion peaks on the high-temperature side are plotted for nylon 12 and 6 in

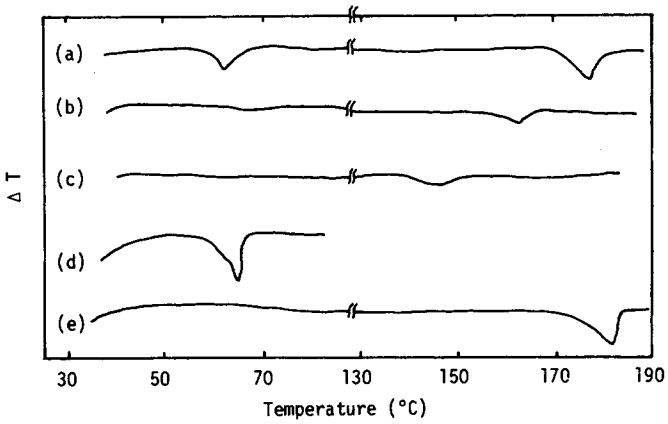

Fig. 1. DSC curves of CPAE (nylon 12/PCL, 37/63 mol %). Curves (a), (b), and (c) show amide–ester interchange reaction times of 30, 120, and 240 min, respectively. Curves (d) and (e) show PCL and nylon 12, respectively.

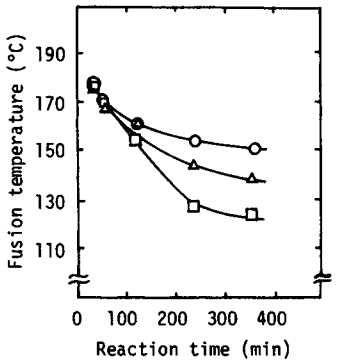

Fig. 2. Fusion temperature depression for nylon 12–PCL systems as a function of the amide–ester interchange reaction time. Molar ratios of nylon 12 and PCL are 20:80 (○), 37:63 (△), and 57:43 (□).

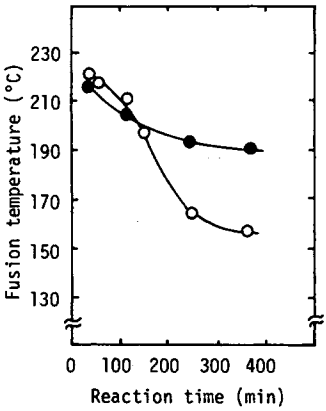

Fig. 3. Fusion temperature depression for nylon 6–PCL systems as a function of the amide–ester interchange reaction time. Molar ratio of nylon 6 and PCL are 70:30 (●) and 50:50 (○).

Figures 2 and 3, respectively, as a function of the amide–ester reaction time. The rate of depression of the fusion peaks slowed gradually with reaction time. This may indicate that the interchange reaction was approaching to equilibrium. Furthermore, the formation of CPAE was confirmed by infrared analysis after the reaction products were fractionated with chloroform, a good solvent of PCL. The mixture of nylon 6 and PCL that was merely blended at 270°C for 10 min underwent almost total quantitative separation into its components after chloroform extraction [Fig. 4(a)]. On the other hand, when this mixture was submitted to the amide-ester interchange reaction for 4 hr, the absorption band at 1724 cm^{-1} due to PCL was detected in the chloroform-insoluble part and the absorption bands at 1642 and 1547 cm^{-1} due to nylon 6 were detected in the chloroform-soluble fraction [Fig. 4(b)]. The weight of the insoluble part increased relative to the blend. The intrinsic viscosity did not change significantly during the course of the amide–ester interchange reaction. (Fig. 5). The above results show that CPAE was formed by the amide–ester interchange reaction between polyamide and polyester.

Length of Polyamide Blocks on CPAE

After hydrolysis of the ester bonds in CPAE by alcoholic alkali at 30°C, the length of the polyamide blocks was measured. The following samples were used: CPAE-1 (reaction time for synthesis, 1 hr) and CPAE-2 (reaction time, 4 hr) composed of nylon 6 and PCL at a 50/50 molar ratio, CPAE-3 (reaction time, 1 hr) and CPAE-4 (reaction time, 4 hr) composed of nylon 12 and PCL at a 50/50 molar ratio, CPAE-5 (reaction time, 4 hr) composed of nylon 6 and PCL at a 20/80 molar ratio, and CPAE-6 (reaction time, 4 hr) composed of nylon 12 and PCL at a 20/80 molar ratio. The infrared spectra after hydrolyzing ester bonds on these CPAEs showed that the ester bonds were almost completely removed, although the absorption band at about 1700 cm^{-1} due to the carboxylic group terminal was detected. The $\overline{M}_n$ of the polyamide blocks of CPAEs 1–6, as estimated by end-group assay, were 3750, 1030, 2870, 850, 830, and 480, respectively. In addition, the molecular weight distribution of these blocks was examined by GPC as shown in Figures 6 and 7. The polymerization degree of each block is

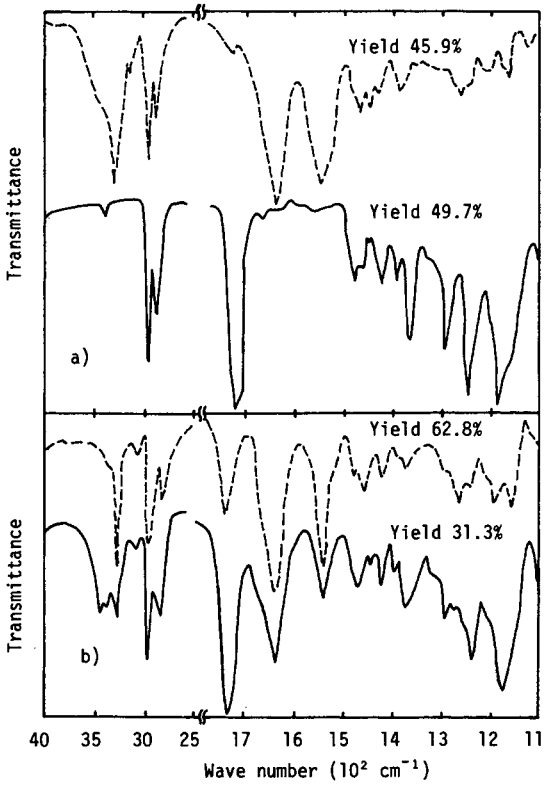

Fig. 4. Infrared spectra of a blend (a) and a CPAE (b) made from nylon 6 and PCL (50/50 mol %). Solid lines and broken lines indicate chloroform-soluble fractions and chloroform-insoluble fractions, respectively.

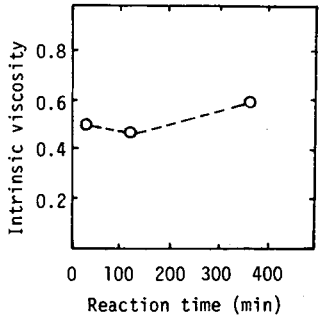

Fig. 5. Effect of the amide–ester interchange reaction time on the intrinsic viscosity of nylon 6–PCL (70/30 mol %) system. Intrinsic viscosity was measured in m-cresol solution at 25°C.

shown in Table 1. These results show that the polyamide blocks in CPAE were shortened with increasing amide–ester interchange reaction time and polyester content and that the polymerization degree of nylon 12 blocks was smaller than that of nylon 6 blocks.

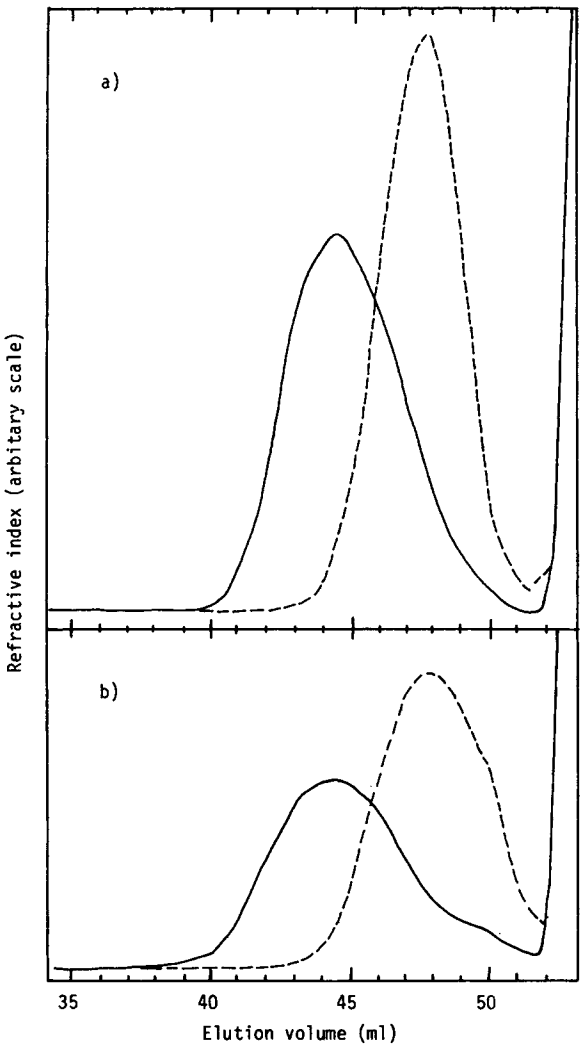

Fig. 6. GPC of the polyamide blocks obtained after hydrolysis of ester bonds on CPAE by alcoholic alkali. The polyamide blocks were derived from (a) nylon 6-PCL (50/50 mol %) and (b) nylon 12–PCL (50/50 mol %) systems. Solid lines and broken lines indicate 1- and 4-hr reaction times for synthesis, respectively. Instrument: model HLC-802R.

Hydrolysis of CPAE by Lipase

The effect of the interchange reaction time on the biodegradability of CPAE was examined. The biodegradability decreased with increasing synthetic reaction time, that is, with the shortening of the polyamide blocks (Figs. 8 and 9). This effect was remarkable in the case of the CPAE containing 50 mol % nylon 6. The biodegradability of the CPAE composed of nylon 12 and PCL decreased rapidly during 30-min reaction time. In order to confirm that the TOC formation in Figs. 8 and Fig. 9 was really based on CPAE and not on unreacted PCL, CPAEs composed of nylon 6 and PCL (molar ratio, 20:80; 4 hr) were fractionated (Fig. 10). The infrared spectra of each fraction (Fig. 11) showed absorption bands of both the amide and ester groups, though the bands of amide group were small

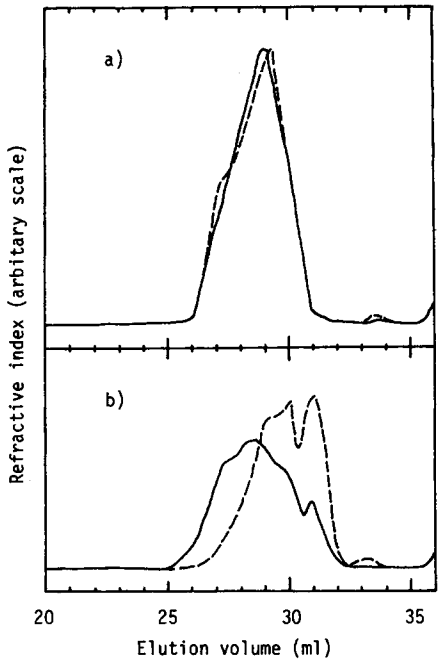

Fig. 7. GPC of the polyamide blocks obtained after hydrolysis of ester bonds in CPAE by alcoholic alkali. The polyamide blocks were derived from (a) nylon 6–PCL (reaction time, 4 hr) and (b) nylon 12–PCL (reaction time, synthesis, 4 hr) systems. Solid lines and broken lines indicate nylon/PCL ratios of 50/50 and 20/80 mol %, respectively. Instrument: model GPC-244.

TABLE I
Polymerization Degree of the Main Component of the Polyamide Blocks of CPAEs 1–6[a]

	CPAE-1	CPAE-2	CPAE-3	CPAE-4	CPAE-5	CPAE-6
Polystyrene used as the standard	94	32	53	16	—	—
Each nylon oligomer used as the standard	—	9 ~ 10	—	2 ~ 6	7 ~ 8	2 ~ 4

[a] The polymerization degree of each block was determined from the position of the peak top on each GPC chromatogram. CPAE-1: nylon 6–PCL (50/50 mol %); reaction time for synthesis, 1 hr. CPAE-2: nylon 6–PCL (50/50 mol %); reaction time for synthesis, 4 hr. CPAE-3: nylon 12–PCL (50/50 mol %); reaction time for synthesis, 1 hr. CPAE-4: nylon 12–PCL (50/50 mol %); reaction time for synthesis, 4 hr. CPAE-5: nylon 6–PCL (20/80 mol %); reaction time for synthesis, 4 hr. CPAE-6: nylon 12–PCL (20/80 mol %); reaction time for synthesis, 4 hr.

in the case of fraction III. Fraction I may contain CPAE and unreacted nylon 6. Fraction III may contain ester-rich CPAE, unreacted PCL, and oligomer of nylon 6. Fraction II contains only CPAE. Fractions I–III were obtained in 13, 20, and 60% yields, respectively. Using 25 mg of fraction II, the biodegradability was examined in a similar manner. Water-soluble TOC corresponding to more than 80% degradation (8640 ppm) was formed. The gas-liquid chromatogram of this TOC after esterification with diazomethane showed two peaks. The retention time of one of the peaks occupying a large portion on the chromatogram was identical with that of methyl ester of authentic ϵ-hydroxycaproic acid (Fig. 12). So it was confirmed that CPAE can be hydrolyzed by lipase.

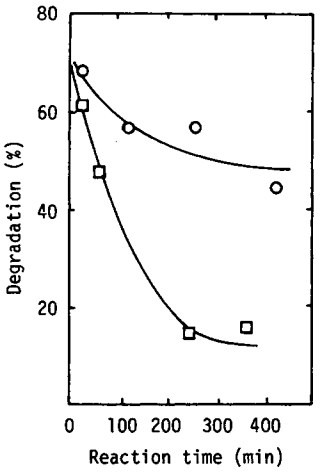

Fig. 8. Effect of the amide–ester interchange reaction time on the biodegradability of CPAE composed of nylon 6 and PCL. Molar ratios of nylon 6 and PCL were 20/80 (○) and 50/50 (□).

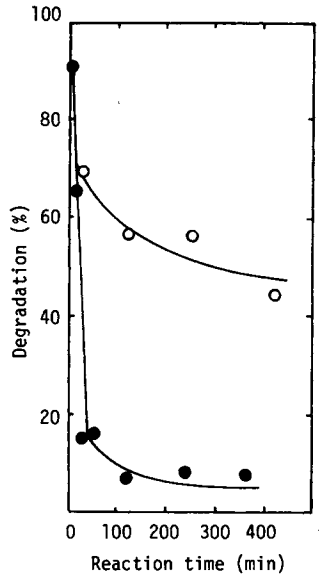

Fig. 9. Effect of the amide–ester interchange reaction time on the biodegradability of CPAE composed of nylons 6 or 12 and PCL (20/80 molar ratio): nylon 6–PCL CPAE (○) and nylon 12–PCL (●).

Effect of Molar Ratio of Polyamide and Polyester on Biodegradability

The effect of the molar ratio of nylon and PCL on the biodegradability of CPAE was examined. The reaction time for each CPAE synthesis was 4 hr. As shown in Figure 13, the biodegradability of CPAE decreased with increasing nylon content. The degradabilities of nylons 11, 12, and 612, in particular, decreased rapidly with increasing nylon content compared with those of nylons 6 and 66. The simple blends of nylon and PCL at 270°C for 10 min retained high biodegradability of PCL. The biodegradability pattern of CPAE made from nylon 69 was intermediate between those of the CPAEs made from nylons 66

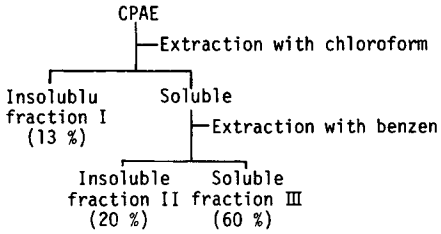

Fig. 10. Fractionation scheme of CPAE composed of nylon 6 and PCL (20/80 molar ratio).

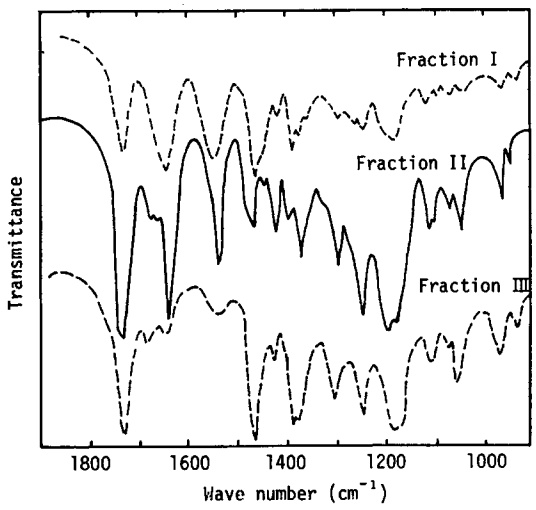

Fig. 11. Infrared spectra of fractions I–III.

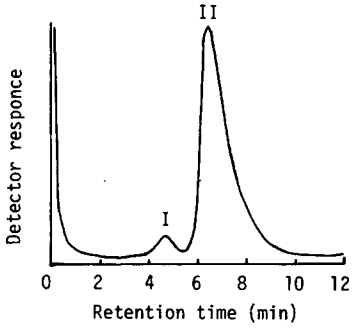

Fig. 12. Gas chromatogram of the hydrolysis products of fractionated CPAE (fraction II) by *Rh. delemar* lipase. Chromatographic conditions: Shimadzu GC-5A (flame ionization) with glass column (1 m × 0.3 cm internal diameter) packed with Tenax GC. Column temperature was 230°C. Flow rate of both nitrogen and hydrogen was 50 ml/min. I, unknown product; II, methylester of ϵ-hydroxycaproic acid.

and 612. Thus it was concluded that the biodegradability of CPAE depended on its composition.

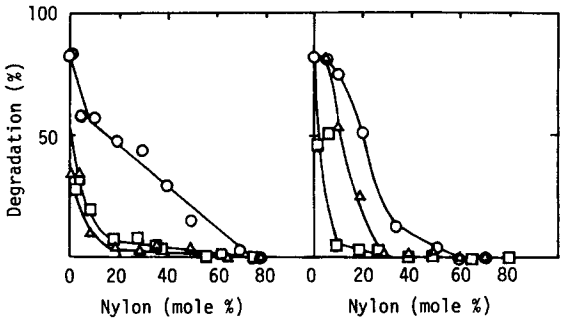

Fig. 13. Effect of the molar ratio of nylon and PCL on biodegradability of CPAE by *Rh. delemar* lipase. The reaction time for each CPAE synthesis was 4 hr. The basic structures of nylon were of two types. One was $[\text{-NH}(\text{CH}_2)_m\text{ CO-}]_n$ (left); the other was $[\text{-NH}(\text{CH}_2)_6\text{NHCO}(\text{CH}_2)_m\text{CO-}]_n$ (right). Left: -○-, nylon 6; -△-, nylon 11; -□-, nylon 12; right: -○-, nylon 66; -△-, nylon 69; -□-, nylon 612.

DISCUSSION

We prepared CPAEs by the amide–ester interchange reaction method. The CPAEs were composed of random blocks of polyamide and polyester. Even if CPAE is synthesized in a given molar ratio of polyamide and polyester, the molar ratios of the individual CPAE molecules will be different from each other. For example, CPAE which was synthesized in a 50/50 molar ratio of polyamide and polyester has a molar ratio distribution from 30/70 to 70/30 (Fig. 14). So to say that CPAE is biodegradable does not necessarily mean that it is completely biodegradable. However, when ester bonds of CPAE are biodegraded, the residue—composed of low-molecular-weight polyamides–can be expected to be biodegradable.[2–4]

It was ascertained that CPAEs made from nylon and PCL were hydrolyzed by lipase. However, the question arises as to why the biodegradation of CPAE is depressed with increasing nylon content (Fig. 13). Blends of nylon 6 and PCL were very well degraded by lipase. It has already been reported that *Rh. delemar* lipase randomly splits the ester bonds of polyester, finally degrading it into the constituent units.[1,7] So it is difficult to explain this result only on the basis of the difference in chemical structure. The effect of hydrogen bonds arising from amide groups are thought to be another factor. The number of hydrogen bonds

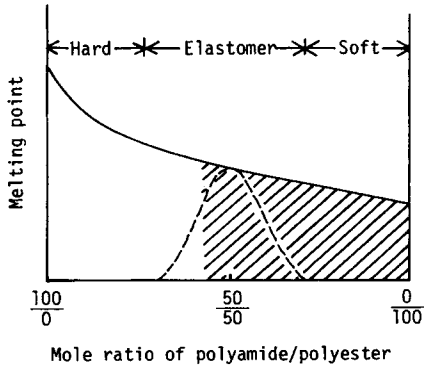

Fig. 14. Schematic diagram of CPAE properties. Shaded part indicates biodegradable CPAE; broken line indicates distribution of molar ratio.

among molecular chains of CPAE increases with increasing nylon content. The effect of hydrogen bonds on the biodegradability may be important because the bond energy is fairly high (ca. 8 kcal/mol) when CPAE is in the particle state in the reaction system. However, it is impossible to explain the phenomenon shown in Fig. 12 on the basis of the number of hydrogen bonds. That is the reason why the depression of biodegradation of CPAE that occurs with increasing nylon content is more remarkable for nylons 11 or 12 than for nylon 6 and more remarkable for nylon 69 or 612 than for nylon 66. It seems that the difference of affinity between the individual nylons and PCL affects the rate of amide–ester interchange reaction. So it is assumed that differences in the distribution of hydrogen bonds in CPAE, that is, differences in the length of the nylon blocks, causes this phenomenon. In fact, the length of nylon blocks of CPAE from nylon 12 was shorter than the length of those from nylon 6 (Table I). From these results it is concluded that the hydrogen bonds among CPAE chains, especially their distribution in CPAE, strongly affected their biodegradation by *Rh. delemar* lipase.

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