Biodegradable Polymers Based on Renewable Resources. V. Synthesis and Biodegradation Behavior of Poly(ester amide)s Composed of 1,4:3,6-Dianhydro-D-glucitol, α-Amino Acid, and Aliphatic Dicarboxylic Acid Units

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ABSTRACT: A series of poly(ester amide)s were synthesized by solution polycondensations of various combinations of *p*-toluenesulfonic acid salts of *O*,*O′*-bis(α-aminoacyl)-1,4:3,6-dianhydro-D-glucitol and bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids with the methylene chain lengths of 4–10. The *p*-toluenesulfonic acid salts were obtained by the reactions of 1,4:3,6-dianhydro-D-glucitol with alanine, glycine, and glycylglycine, respectively, in the presence of *p*-toluenesulfonic acid. The polycondensations were carried out in *N*-methylpyrrolidone at 40°C in the presence of triethylamine, giving poly(ester amide)s having number-average molecular weights up to 3.8 $\times 10^4$. Their structures were confirmed by FTIR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Most of these poly(ester amide)s are amorphous, except those containing sebacic acid and glycine or glycylglycine units, which are semicrystalline. All these poly(ester amide)s are soluble in a variety of polar solvents such as dimethyl sulfoxide, *N*,*N*-dimethylformamide, 2,2,2-trifluoroethanol, *m*-cresol, pyridine, and trifluoroacetic acid. Soil burial degradation tests, BOD measurements in an activated sludge, and enzymatic degradation tests using *Porcine pancreas* lipase and papain indicated that these poly(ester amide)s are biodegradable, and that their biodegradability markedly depends on the molecular structure. The poly(ester amide)s were, in general, degraded more slowly than the corresponding polyesters having the same aliphatic dicarboxylic acid units, both in composted soil and in an activated sludge. In the enzymatic degradation, some poly(ester amide)s containing dicarboxylic acid components with shorter methylene chain lengths were degraded more readily than the corresponding polyesters with *Porcine pancreas* lipase, whereas most of the poly(ester amide)s were degraded more rapidly than the corresponding polyesters with papain. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2721–2734, 2001

Key words: biodegradable polymer; poly(ester amide); polycondensation; 1,4:3,6-dianhydro-D-glucitol; enzymatic degradation

INTRODUCTION

Recently, worldwide concern for the preservation of the global environment is becoming increasingly stronger day after day. The global environmental issues, however, involve numerous complicated factors, and the solutions cannot be straightforward. From the view point of polymer science and industries, use of biodegradable polymers, together with more effective and extensive recycling of waste plastics, should be promoted as much as possible to prevent the increasing contamination of the global environment. Therefore, development of biode-

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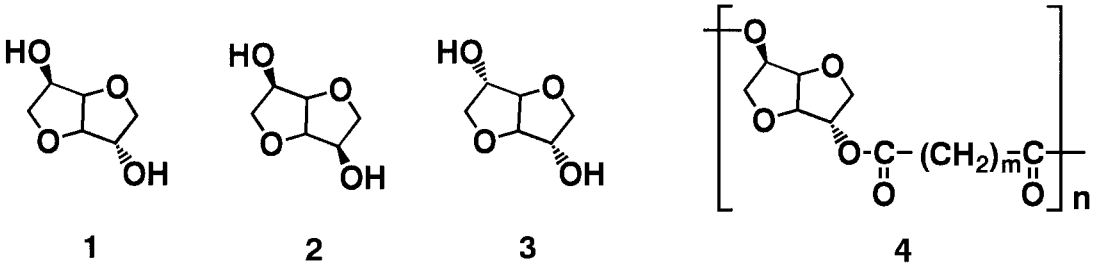
gradable polymers applicable for practical uses is an urgent matter.

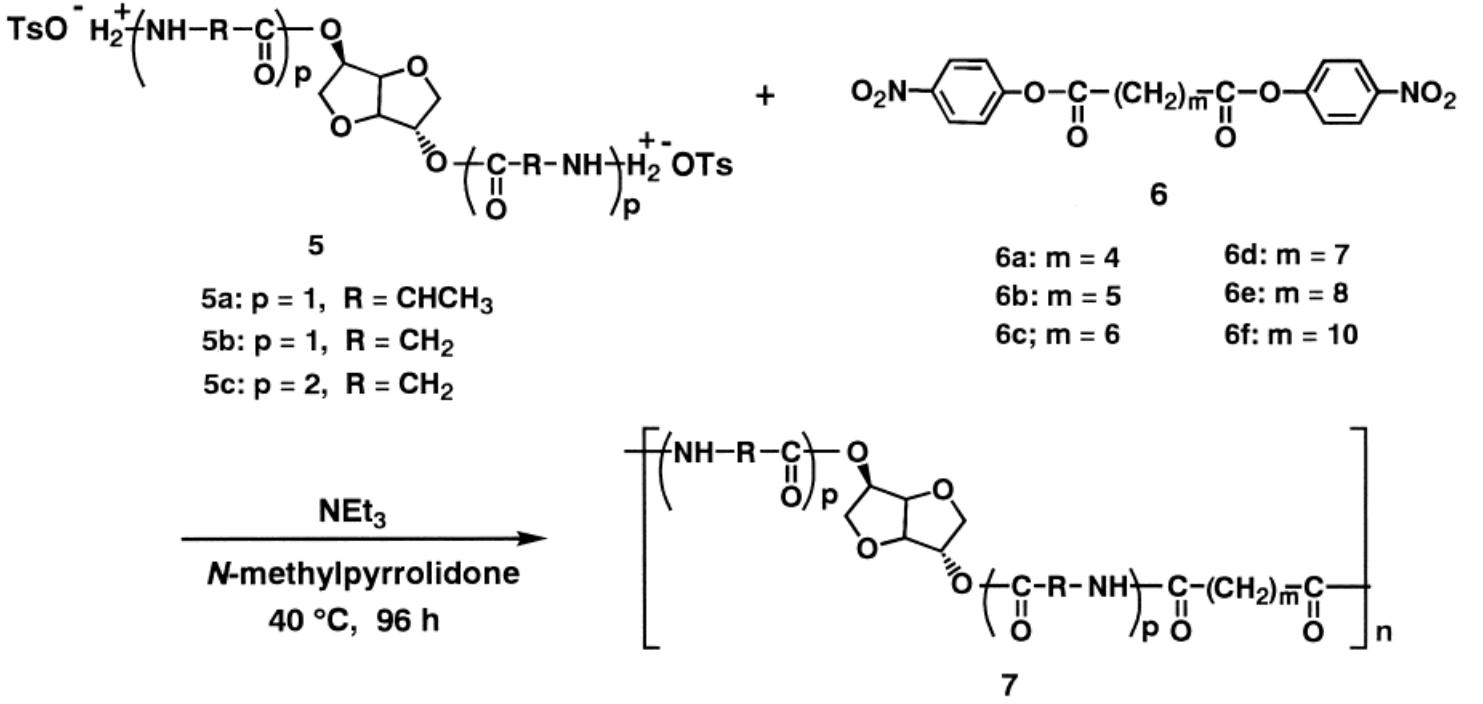
It is well known that aliphatic polyesters are biodegradable, and numerous original articles and review articles have been published so far.[1–5] At present, some biodegradable polyesters are commercially available, but their still insufficient thermal and/or mechanical properties limit their broad use. On the other side, aliphatic polyamides are not readily biodegradable, although there are a few reports demonstrating their biodegradability.[6–12] Compared to aliphatic polyesters, aliphatic polyamides possess higher thermal stability, higher modulus, and higher tensile strength. Therefore, it is reasonable to combine the favorable properties of these two classes of polymers to produce new polymeric materials possessing not only good biodegradability but also good materials and processing properties.

A number of articles concerning syntheses of poly(ester amide)s have been reported in the literature, and some of them have dealt with their biodegradation. Saotome et al.[13,14] reported the enzymatic degradation of poly(ester amide)s derived from 1,2-ethanediol, adipic acid, and different amino acids. They showed that only the polymers containing glycine were not degraded by any of the tested proteolitic enzymes (chymotripsin and esterase) and that inclusion of phenylalanine in the glycine-derived poly(ester amide)s enhanced their degradability with chymotripsin. Puiggali et al.[15–17] synthesized two poly(ester amide)s from sebacic acid, 1,12-dodecandiol, and alanine in both the L-configuration and the racemic D,L-mixture. Their biodegradation studies, using different enzymes, demonstrated that papain was the most effective, although the degradation rate was dependent on the stereochemical composition of the polymer. Nagata[18] derived poly(ester amide)s from 1,6-hexanediol diester of L- and D-alanine and sebacoyl chloride, and found that the degradation with proteolytic enzymes (proteinase K, papain, and α-chymotripsin) was not caused by hydrolysis of the semipeptide linkage but of the ester linkage. Shirahama et al.[19] prepared cyclic depsipeptide/ϵ-caprolactone copolymers, and reported that noncrystalline parts containing more depsipeptide in the copolymers were degraded preferentially by proteinase K. Katsarava et al.[20] synthesized a series of poly(ester amide)s from α-amino acids, α,ω-diols, and aliphatic dicarboxylic acids, and assessed the effect of the building blocks of the polymers on their biodegradation properties. Very recently, Gomurashvili et al.[21] reported the synthesis of a series of poly(ester amide)s from dianhydrohexitols, hydrophobic α-amino acids, and aliphatic dicarboxylic acids, and their enzymatic degradation using α-chymotripsin and lipase.

In concert with the depletion of oil resources, increasingly greater attention has been directed to effective utilization of plant-biomass as alternative, renewable resources that can be steadily supplied and used for polymer syntheses. We also have been investigating polymer syntheses from renewable resources[22–24] and biopolymer–synthetic polymer hybrids as well.[25–28] In particular, we have been engaged in biodegradable polymer syntheses using three stereoisomeric 1,4:3,6-dianhydrohexitols, that is, 1,4:3,6-dianhydro-D-glucitol (**1**), 1,4:3,6-dianhydro-D-mannitol (**2**), and 1,4:3,6-dianhydro-L-iditol (**3**).[29–33] Thus, a series of polyesters (**4**) and copolyesters were synthesized by polycondensations of 1,4 : 3,6-dianhydrohexitols with aliphatic dicarboxylic acid derivatives. Polyesters and copolyesters containing **1–3** and 1,1-bis(5-carboxy-2-furyl)ethane units were also synthesized.[32,33] Most of these polyesters were found to be biodegradable by soil burial degradation tests, BOD measurements in an activated sludge, and enzymatic degradation tests.



As an extension of the biodegradable polymer syntheses based on plant-biomass resources, we want to describe here synthesis and biodegradation behavior of a series of poly(ester amide)s (**7**)



Code No.	p	R	m	Code No.	p	R	m
7a	1	$CHCH_3$	4	7e	1	$CHCH_3$	8
7b	1	$CHCH_3$	5	7f	1	$CHCH_3$	10
7c	1	$CHCH_3$	6	7g	1	CH_2	8
7d	1	$CHCH_3$	7	7h	2	CH_2	8

Scheme 1 Synthesis of poly(ester amide)s **7a–7h** by polycondensations of various combinations of *O*,*O*′-bis(α-aminoacyl)-1,4 : 3,6-dianhydro-D-glucitol **5a–5c** with bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids **6a–6f.**

composed of 1,4:3,6-dianhydro-D-glucitol (**1**) readily available from D-glucose, α-amino acid, and aliphatic dicarboxylic acid units (Scheme 1).

EXPERIMENTAL

Materials

Commercially available 1,4:3,6-dianhydro-D-glucitol (**1**) was purified by repeated recrystallization from chloroform. *p*-Toluenesulfonic acid salts of *O*,*O*′-bis(α-aminoacyl)-1,4:3,6-dianhydro-D-glucitol (**5a–5c**) were prepared by the reactions of **1** with alanine, glycine, and glycylglycine, respectively, in refluxing toluene in the presence of *p*-toluenesulfonic acid monohydrate with a Dean-Stark equipment. Bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids (**6a–6f**) were prepared by the reaction of the corresponding acid chlorides with *p*-nitrophenol in acetonitrile in the presence of pyridine. They were purified by recrystallization from ethyl acetate twice.

Polycondensation

Polycondensations of various combinations of **5a–5c** with **6a–6f** were carried out in *N*-methylpyrrolidone in the presence of triethylamine at 40°C for 96 h. The reaction mixture was poured into a large volume of acetone or a mixture of ethyl acetate and hexane (1 : 1, v/v) to precipitate polymer. It was thoroughly washed with water and dried under reduced pressure to a constant weight.

Characterization

Molecular weights of the poly(ester amide)s were estimated by size-exclusion chromatography (SEC) using dimethyl sulfoxide containing 30 m*M* LiCl and 1% (v/v) chloroform as eluent and poly(8-oxa-6-azabicyclo[3.2.1]octan-7-one) as a reference at 50°C. ^{1}H and ^{13}C-NMR spectra of poly(ester amide)s were taken by a VARIAN 3-2 GEMINI 2000, operating at 300 MHz, and a Bruker ARX 400, operating at 400 MHz on solutions in dimethyl sulfoxide-d_6 using tetramethylsilane as an internal reference. Surfaces of poly(ester amide) films after soil burial tests were observed with a JOEL JSM-F7 scanning electron microscope or an Hitachi S-2150 scanning electron microscope. Thermal transition temperatures of the poly(ester amide)s were determined with Seiko-Instruments DSC6100 and 6200 differential scanning calorimeters at a heating rate of 10°C/min. Total organic carbon concentrations (TOC) in aqueous solutions produced by enzymatic degradation of the polyesters were determined with a Shimadzu TOC-500A instrument.

Soil Burial Degradation

Soil burial degradation tests were undertaken on thin films (10 × 10 mm, thickness 200 μm). The films were buried in soil in a desiccator, in which the relative humidity was adjusted to 70–80% with a saturated aqueous solution of ammonium nitrate. The soil, which had been composted for more than 10 years, was obtained from Nagoya University farm. The desiccator was placed in a room thermostated at 27°C. After designated times, the films or disks were taken out, washed with water, and dried. When soil adhered to the samples could not be removed by washing, the samples were extracted with 2,2,2-trifluoroethanol, and the soil was separated by filtration. After evaporation of the solvent from the filtrate, the residue was dried to a constant weight under reduced pressure. The molecular weights of the recovered poly(ester amide)s were determined by SEC.

Enzymatic Degradation

The enzymes used in the present investigation were *Porcine pancreas* lipase (Sigma Chemical) and papain (Sigma Chemical). They were used as supplied. A powdery sample (25 mg) was placed in each test tube, and a phosphate buffer solution of pH 7.0 (10 mL) and enzyme (250 units) were added to the test tube. The test tube, with a screw cap, was incubated with constant shaking at 80 strokes per minute for 24 h at 37°C. As blank tests, test tubes containing either only the sample or the enzyme were shaken under the same conditions. 1*N* Hydrochloric acid (3 mL) was added to each mixture, and the mixture was filtered through a Millipore filter. An aliquot of 100 μL of the filtrate was taken out and diluted with water to 10 mL. Total organic carbon concentrations (TOC) in aqueous solutions, produced by enzymatic degradation of the poly(ester amide)s, were determined with a Shimadzu TOC-500A instrument.

Biochemical Oxygen Demand (BOD) Test in an Activated Sludge

An activated sludge was prepared so that the suspension concentration was 30 mg/L according to Japan industrial standard JIS K 6950, using

Table I Synthesis of Poly(ester amide)s by Polycondensations of bis(α-Amino acid) Esters of 1,4 : 3,6-Dianhydro-D-glucitol 5a–5c **with bis(*p*-Nitrophenyl) Esters of Aliphatic Dicarboxylic Acids** 6a–6f[a]

Diamine	g	Diester	g	NEt_3, mL	NMP, mL	Polymer	Yield, %	M_n[b] $\times 10^{-3}$	M_w/M_n[b]	T_g[c] °C	T_m[c] °C	ΔH_f[c,d] J/g
5a	1.10	**6a**	0.68	0.49	2.9	**7a**	60	14.9	1.4_4	56	—	—
5a	0.88	**6b**	0.56	0.39	2.3	**7b**	71	10.6	1.5_4	45	—	—
5a	0.64	**6c**	0.42	0.29	1.7	**7c**	85	11.2	1.4_2	57	—	—
5a	1.00	**6d**	0.68	0.45	1.7	**7d**	76	20.7	1.4_8	46	—	—
5a	1.47	**6e**	1.03	0.66	1.2	**7e**	83	37.8	1.4_0	57	—	—
5a	1.03	**6f**	0.77	0.46	2.7	**7f**	67	15.1	1.5_5	28	—	—
5b	2.42	**6e**	1.78	1.12	6.7	**7g**	90	19.4	2.0_6	66	164	31
5c	1.17	**6e**	0.72	0.46	2.7	**7h**	89	26.7	1.7_0	47	124	19

[a] Equimolar monomers were charged; temperature, 40°C; time, 96 h.
[b] Determined by SEC in dimethyl sulfoxide + 30 m*M* LiCl + 1 vol % $CHCl_3$ at 50°C (poly(8-oxa-6-azabicyclo[3.2.1]octan-7-one) standard).
[c] Determined by DSC (heating rate, 10°C/min).
[d] Heat of fusion.

an activated sludge taken from a sewage plant in Meito-ku, Nagoya. A film sample (thickness, 200 μm; 10 mg) and the activated sludge (150 mL) were taken in a bottle, and the oxygen consumption was measured at 25°C for 4 days by a TITEC, BOD Tester 200F. The BOD-based biodegradability was estimated by the percent of the consumed amount of oxygen, corrected for a blank test to the theoretical amount of oxygen required for complete oxidation of the sample.

RESULTS AND DISCUSSION

Preparation of Poly(ester amide)s

Poly(ester amide)s were prepared in *N*-methylpyrrolidone at 40°C in the presence of triethylamine by solution polycondensations of various combinations of *p*-toluenesulfonic acid salts of *O*,*O*′-bis(α-aminoacyl)-1,4:3,6-dianhydro-D-glucitol **5a–5c** with six different bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids **6a–6f** (Scheme 1). Table I presents some of the results of the polycondensations. Polymers with number-average molecular weights up to 3.8×10^4 were obtained. In contrast, our attempts to polymerize **5a–5c** with the corresponding acid dichlorides instead of bis (α-amino acid) esters **6a–6f** in chloroform at 27°C in the presence of pyridine gave, in most cases, polymers having number-average molecular weights of less than 10,000.

Poly(ester amide)s **7g** and **7h** are semicrystalline polymers having melting points at 164 and 124°C (DSC), respectively, whereas the other poly(ester amide)s are amorphous. However, judging from the small values of heat of fusion, together with X-ray diffraction measurements, the crystallinity of these two polymers is low (<30%). Most of the poly(ester amide)s show a glass transition in a rather narrow temperature range from 40 to 70°C. Polyester **4** ($m = 8$), consisting of **1** and sebacic acid units, shows its glass transition temperature and melting point at −10 and 61°C, respectively. Therefore, the observed data clearly demonstrate that the incorporation of α-amino acid units into the polymer chain significantly improves the thermal properties.

All these poly(ester amide)s are soluble in a variety of polar solvents, including *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, dimethyl sulfoxide, 2,2,2-trifluoroethanol, pyridine, trifluoroacetic acid, and *m*-cresol. They are insoluble or partly insoluble in chloroform, and insoluble in acetonitrile, tetrahydrofuran, 1,4-dioxane, ethyl acetate, and toluene, although polyester **4** ($m = 8$), having an M_n of 28×10^3, is soluble in all these solvents.

These polymers were confirmed to be poly(ester amide)s of regular structure by ^{1}H-NMR, ^{13}C-NMR, and FTIR spectroscopy. A typical ^{13}C-NMR spectrum of poly(ester amide) is shown in Figure 1. It is to be noted here that there appears to be two pairs of carbonyl carbon signals at δ 173.89 and 173.74 ppm (ester carbonyl), and δ 170.48 and 170.38 ppm (amide carbonyl). The appearance of these pairs of peaks arise from the nonequivalence of the C—O bonds at C(2) and C(5) of a 1,4:3,6-dianhydro-D-glucitol unit, that is, the former is in the exo-position, whereas the latter in

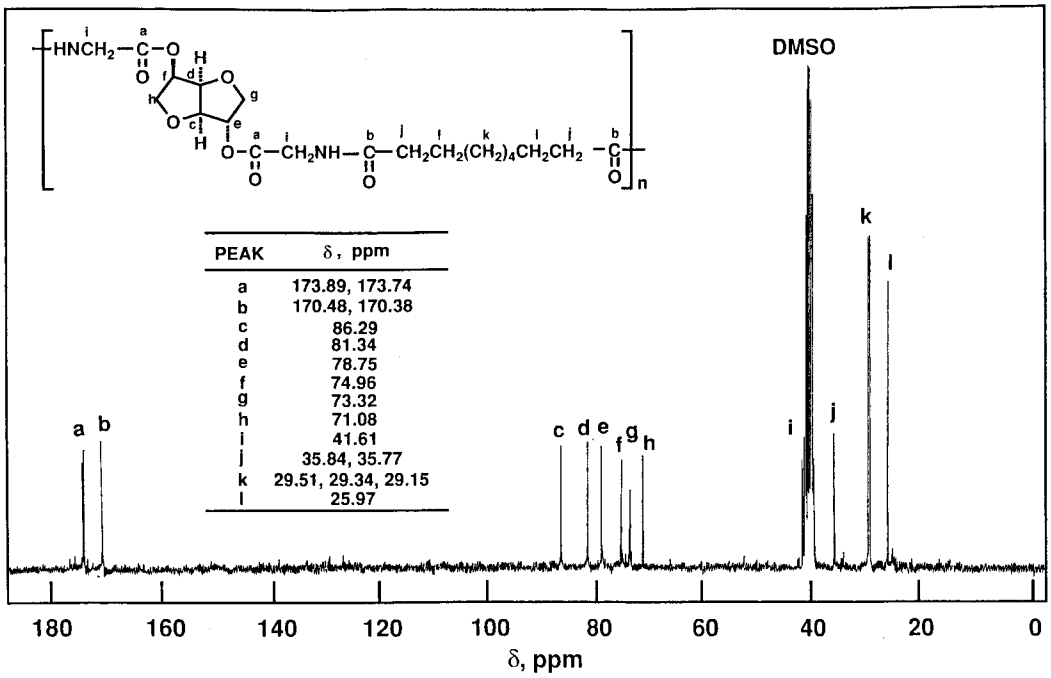


Figure 1 ^{13}C-NMR spectrum of poly(ester amide) **7g.** Solvent, dimethyl sulfoxide-d_6; concentration, 10%; room temperature; 75 MHz; tetramethylsilane.

the endo-position. All other signals were unequivocally assigned as shown in the figure, and there is no indication that scrambling of the ester and amide linkages occurs during the polycondensation.

Soil Burial Degradation

The soil burial degradation test was carried out at 27°C in the soil, which had been composted for more than 10 years at the Nagoya university farm. Figure 2 shows the results on the soil burial degradation of the films of three poly(ester amide)s **7e, 7g,** and **7h** differing in α-amino acid components and having sebacic acid units as a common dicarboxylic acid component. The relevant data of the corresponding polyester **4** (m = 8) are also shown in Figure 2 for comparison. Polyester **4** was degraded most rapidly, and the film completely disappeared after soil burial for 24 days. Among the three poly(ester amide)s, **7e** was degraded faster. The weight recovery was 10% after 40 days, and the film disappeared after 50 days. In contrast, poly(ester amide)s **7g** and **7h** underwent slower degradation, and the weight recovery was 36% after 90 days for **7g** and 50% after 80 days for **7h,** respectively. Presumably, the higher proportion of the amide groups of **7h** is responsible for the slower degradation rate. The number-average molecular weights of the residual polymers decreased gradually with prolonged soil burial time, suggesting that hydrolysis of poly(ester amide) chains may occur, to some extent, during soil burial test.

Figure 3 shows an SEM photograph of the film of poly(ester amide) **7c** after soil burial for 30 days. The photograph shows that the film surface was eroded by filamentous fungi. Filamentous fungi were observed also in the SEM photographs of the films of **7g** and **7h.** Figure 4 is an SEM photograph of the film of poly(ester amide) **7g** in a higher magnification after soil burial for 50 days, showing that the film surface under the colony of sporangia was eroded by actinomycetes. These photographs demonstrate that the poly(ester amide)s containing **1,** α-amino acid, and sebacic acid units are biodegraded in soil. Presumably, the apparently lower degradability of **7g** and **7h** compared to that of **7e** is ascribable to the semicrystallinity of the former two poly(ester amide)s.

Figure 5 compares the soil burial degradation of three poly(ester amide)s **7d, 7e,** and **7f** containing L-alanine units as a common α-amino acid component and having dicarboxylic acid units with different methylene chain lengths. Interestingly, poly(ester amide)s **7d** and **7f** with the methylene chain lengths of 7 and 10, respectively, were degraded more slowly than **7e** having the methylene chain length of 8. Thus, the weight recovery

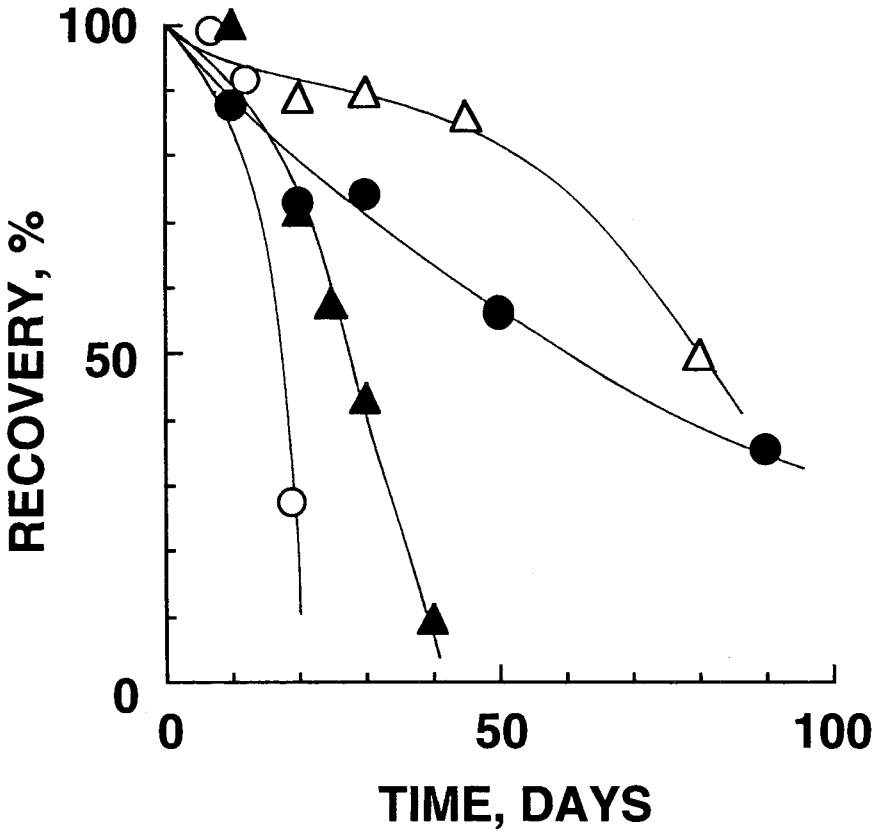


Figure 2 Recovery (wt %) of poly(ester amide)s **7e, 7g, 7h,** and polyester **4** ($m = 8$) in soil burial test. Conditions: film thickness, 200 μm; composted soil, pH 6.8; temperature, 27°C; humidity, 70—80%. ▲ : **7e,** ● : **7g,** △ : **7h,** ○ : **4** ($m = 8$).

after 60 days was 45% for **7d** and 38% for **7f,** while **7e** was completely degraded before 60 days. The reason for the faster degradation behavior of **7e** remains to be clarified. Although the data are not shown in Figure 5, all the films of poly(ester amide)s **7a–7c** with the methylene chain lengths of 4–6, respectively, had disappeared after soil burial for 20 days. Poly(ester amide)s **7a–7c** are of higher hydrophilicity, and hence, we cannot exclude the possibility that hydrolysis by moisture apparently accelerated their degradation in the soil.

Degradation in an Activated Sludge

BOD measurements on the poly(ester amide)s were carried out in an activated sludge according to JIS K6950. The BOD-based biodegradability is defined in the Experimental section. The results on poly(ester amide)s **7e, 7g,** and **7h,** along with the data on polyester **4** ($m = 8$), are shown in Figure 6. The polyester was most readily degraded in the activated sludge, and its BOD biodegradability reached 40% after 28 days. The poly(ester amide)s, particularly **7e,** slowly underwent degradation in the activated sludge. Thus, their BOD degradability after 28 days was 33% for **7g** containing glycine as an α-amino acid unit, 23% for **7h** containing glycylglycine, and only 5% for **7e** containing L-alanine.

The BOD biodegradability values of these poly(ester amide)s were calculated on the assumption that all the amide linkages in the samples are hydrolyzed and oxidized to nitric acid. Actually, however, it would be more likely that only a part of amide linkages are converted to nitric acid, because only limited kinds of bacteria are capable of oxidizing ammonia to nitric acid, and also the conditions suitable for nitrifying bacteria to work actively are restricted. Therefore, it seems reasonable to assume that the actual BOD-biodegradability values are somewhat higher than those shown in Figure 6.

The order of the BOD biodegradability of the three poly(ester amide)s (**7g** > **7h** > **7e**) differs from the trend in the soil burial degradation (**7e** > **7g** > **7h**). Micro-organisms in an activated sludge are

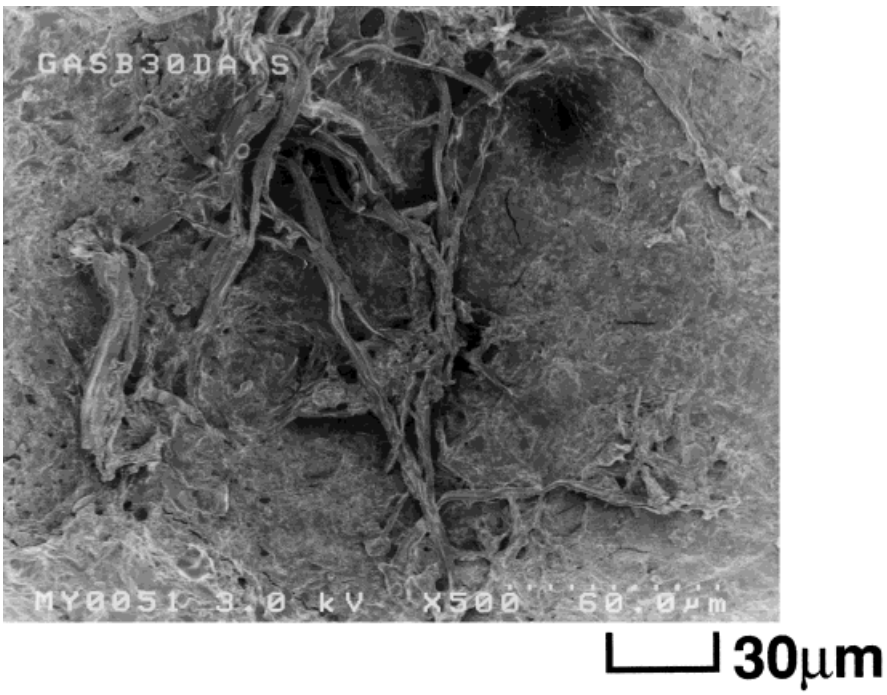


Figure 3 SEM photograph of poly(ester amide) **7e** recovered after soil burial for 30 days. Conditions; film thickness, 200 μm; composted soil, pH 6.8; temperature, 27°C; humidity, 70–80%.

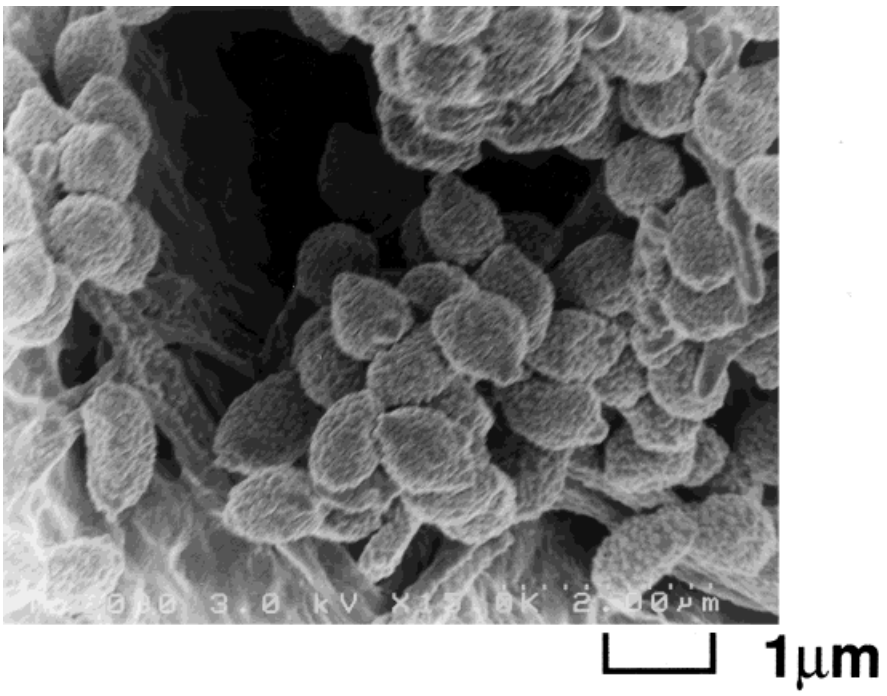


Figure 4 SEM photograph of poly(ester amide) **7g** recovered after soil burial for 50 days. Conditions; film thickness, 200 μm; composted soil, pH 6.8; temperature, 27°C; humidity, 70–80%.

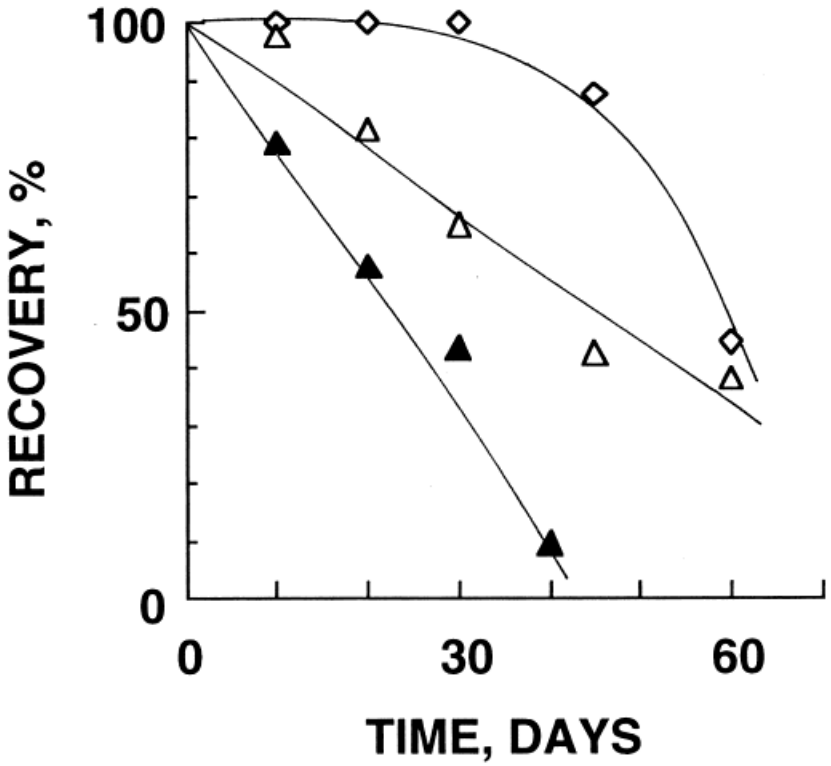


Figure 5 Recovery (wt %) of poly(ester amide)s **7d–7f** in soil burial test. Conditions: film thickness, 200 μm; composted soil, pH 6.8; temperature, 27°C; humidity, 70–80%. ◇ : **7d,** ▲ : **7e,** △ : **7f.**

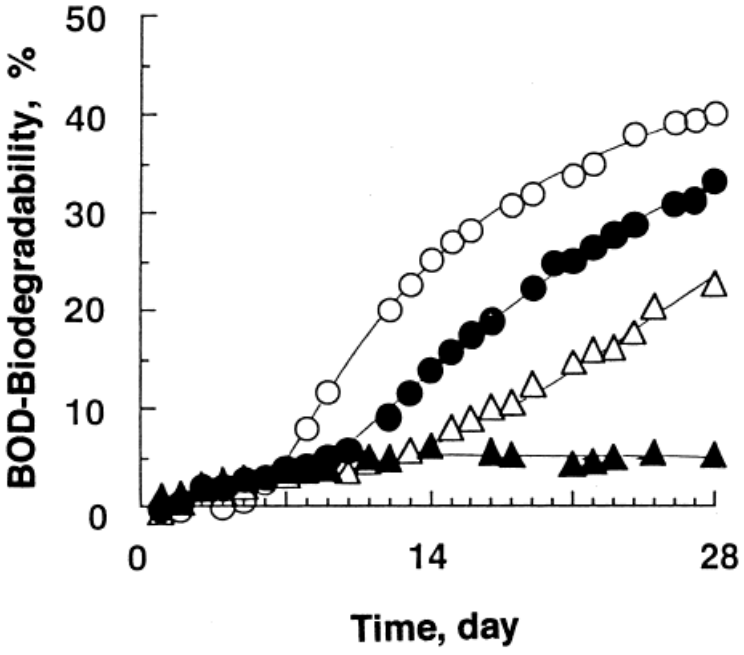


Figure 6 Biodegradation of poly(ester amide)s **7e, 7g, 7h,** and polyester **4** ($m = 8$) in an activated sludge. Conditions: sample 15 mg: activated sludge, 150 mL; temperature, 25°C. ▲ : **7e,** ● : **7g,** △ : **7h,** ○ : **4** ($m = 8$).

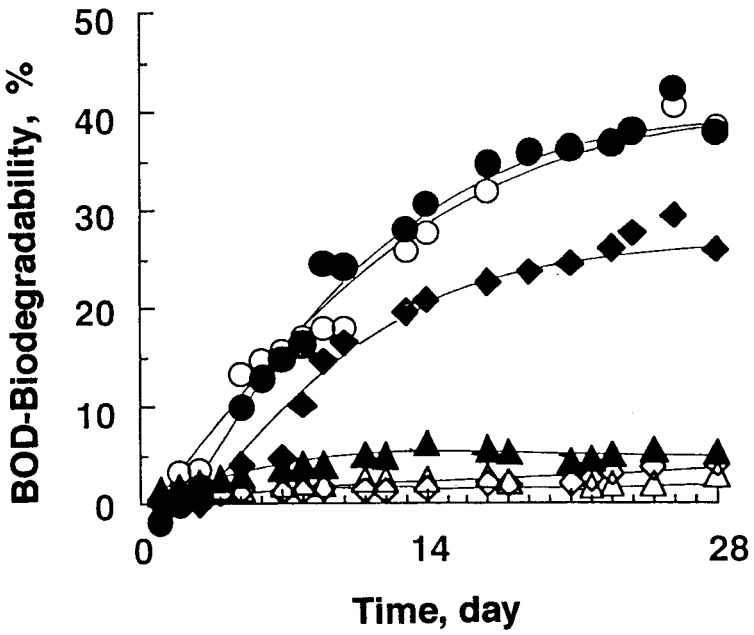


Figure 7 Biodegradation of poly(ester amide)s **7a–7f** in an activated sludge. Conditions: sample 15 mg: activated sludge, 150 mL; temperature, 25°C. ● : **7a,** ○ : **7b,** ◆ : **7c,** △ : **7d,** ▲ : **7e,** ◇ : **7f.**

mainly bacteria, and in addition, the kinds differ from those present in soil. As described above, poly(ester amide) **7e** was degraded chiefly by filamentous fungi in the composted soil, and hence, it is understandable that **7e** was less susceptible to degradation in the activated sludge.

Figure 7 shows the results on the degradation of a series of poly(ester amide)s **7a–7f** containing L-alanine as a common α-amino acid component and differing in the dicarboxylic acid components. Poly(ester amide)s **7a–7c** with the methylene chain lengths of 4, 5, and 6 showed the BOD biodegradability values of 38, 38, and 25%, respectively, after 28 days. In sharp contrast, the BOD-biodegradability values for poly(ester amide)s **7d–7f** were less than 5%. This trend differs considerably from that found in the BOD measurements of the corresponding polyesters **4.**[33] In the polyester series, the maximum BOD biodegradability of 62% was found in polyester **4** (m = 6) with the methylene chain length of 6 after 28 days, and polyesters **4** with the methylene chain lengths of 7, 8, and 10 also showed BOD biodegradability (30–40%) comparable to that of polyesters with the methylene chain lengths of 4 and 5.

As the molecular weights of **7a–7c** used for the BOD measurement were nearly 10,000, appreciably lower than those of the other poly(ester amide)s ($M_n > 20 \times 10^3$), the lower molecular weights of **7a–7c** might be, at least partly, responsible for the faster degradation. However, even if we take this factor into consideration, we may safely say that the BOD degradability of these poly(ester amide)s is markedly dependent, not only on the kind of amino acids, but also on the methylene chain length of the dicarboxylic acid components.

Enzymatic Degradation of Poly(ester amide)s

Enzymatic degradation of the poly(ester amide)s was monitored by TOC measurements of the phosphate buffer solutions containing water-soluble degradation products. The TOC measurements were carried out three times on each combination of a sample and an enzyme, and the average value was represented as TOC. In general, the error limit of the TOC measurement was within 10%. The TOC values were corrected for the concurrent hydrolysis by subtracting the TOC value in the blank test without enzyme from the observed values with enzyme.

Figure 8 presents some of the results on the enzymatic degradation of poly(ester amide)s **7e, 7g,** and **7h** using two enzymes, along with those for polyester **4** (m = 8) for comparison. With *Porcine pancreas* lipase, polyester **4** was most readily degraded, followed by poly(ester amide)s **7e** and **7h,** whereas poly(ester amide) **7g** contain-

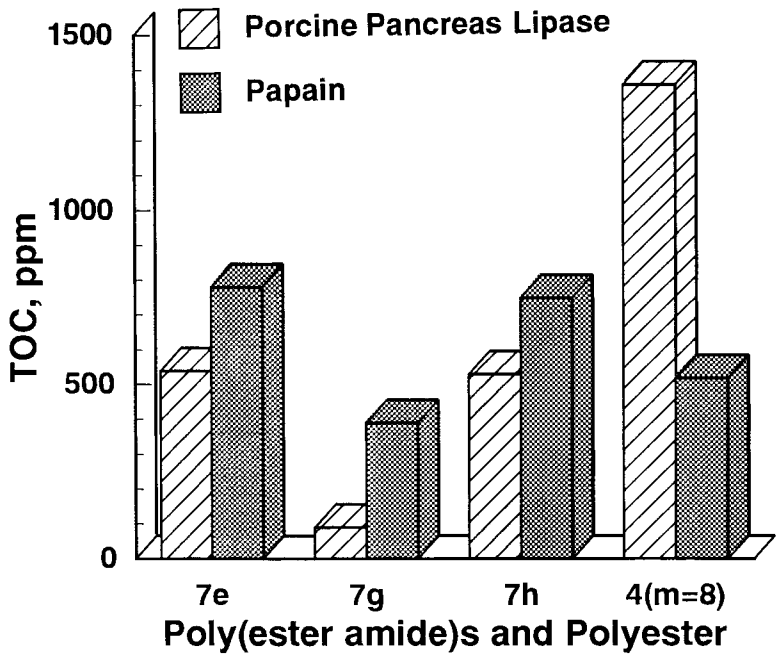


Figure 8 Enzymatic degradation of poly(ester amide)s **7e, 7g, 7h,** and polyester **4** (m = 8) by *Porcine pancreas* lipase and papain. Conditions: enzyme, 250 units; sample, 25 mg; phosphate buffer, pH 7.0, 2 mL; incubated at 80 strokes/min at 37°C for 24 h.

ing glycine as an α-amino acid component was least degraded. The lower degradability of **7g** might not be ascribable to its higher crystallinity, because it was reported that the enzymatic action of *Porcine pancreas* lipase is not affected very much by crystallinity.[34] In contrast, when papain, a protease, was used, poly(ester amide)s **7e** and **7h** containing L-alanine and glycylglycine, respectively, showed higher TOC values than polyester **4.** Poly(ester amide) **7g** showed the lowest TOC value also with this enzyme, although the differences in TOC values among these four substrates are not very significant.

When lower molecular weight samples (M_n = 8.0×10^3–8.8×10^3) were used for the enzymatic degradation, the TOC values increased to two to four times higher than those for the higher molecular weight samples ($M_n > 20 \times 10^3$) presented in Figure 8. It is to be noted here that α-chymotrypsin from bovine pancreas was completely ineffective for the degradation of these poly(ester amide)s and polyester. This is understandable, because α-chymotrypsin is a protease, but unlike papain, it is a highly substrate-specific enzyme that preferentially cleaves the C-terminal bond of aromatic amino acids in various peptides under physiological conditions.[35]

Enzymatic degradation of poly(ester amide)s **7a–7f,** containing L-alanine as a common α-amino acid component and having different methylene chain length in the dicarboxylic acid component, were investigated with *Porcine pancreas* lipase and papain as enzymes. The results are graphically represented in Figures 9 and 10, respectively, along with the results on polyesters **4** for comparison. With *Porcine pancrease* lipase, poly(ester amide)s **7c–7f** with the methylene chain lengths of 6 or higher showed lower degradability than the corresponding polyesters **4** (m = 6, 7, 8, and 10) as judged from the TOC values (Fig. 9). In sharp contrast, poly(ester amide)s **7a** and **7b** with the methylene chain length of 4 and 5, respectively, showed higher degradability than the corresponding polyesters **4** (m = 4 and 5).

As we discussed in the previous article,[33] a relatively large spacing between the ester linkages and proper hydrophilic and hydrophobic balance of polyesters **4** are required for the effective approach of the enzyme to the polyester substrates and for the effective enzymatic action. Therefore, the placement of an additional L-alanine unit between 1,4:3,6-dianhydro-D-glucitol and aliphatic dicarboxylic acid units seems to bring an effect similar to the elongation of the methylene chain length.

As expected, poly(ester amide)s showed higher degradability than the corresponding polyesters when they are exposed to a phosphate-buffered

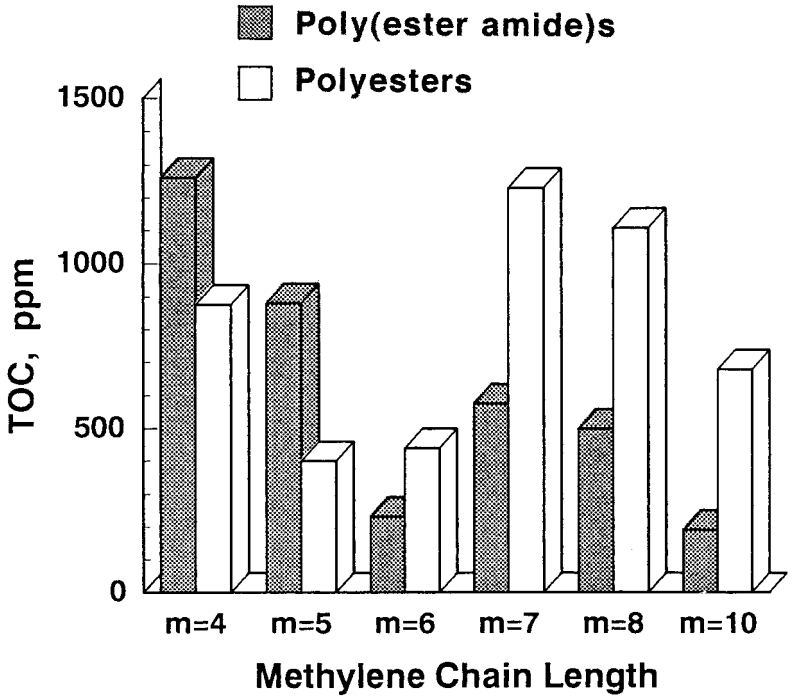


Figure 9 Enzymatic degradation of poly(ester amide)s **7a–7f** and polyesters **4** by *Porcine pancreas* lipase. Conditions: enzyme, 250 units; sample, 25 mg; phosphate buffer, pH 7.0, 2 mL; incubated at 80 strokes/min at 37°C for 24 h.

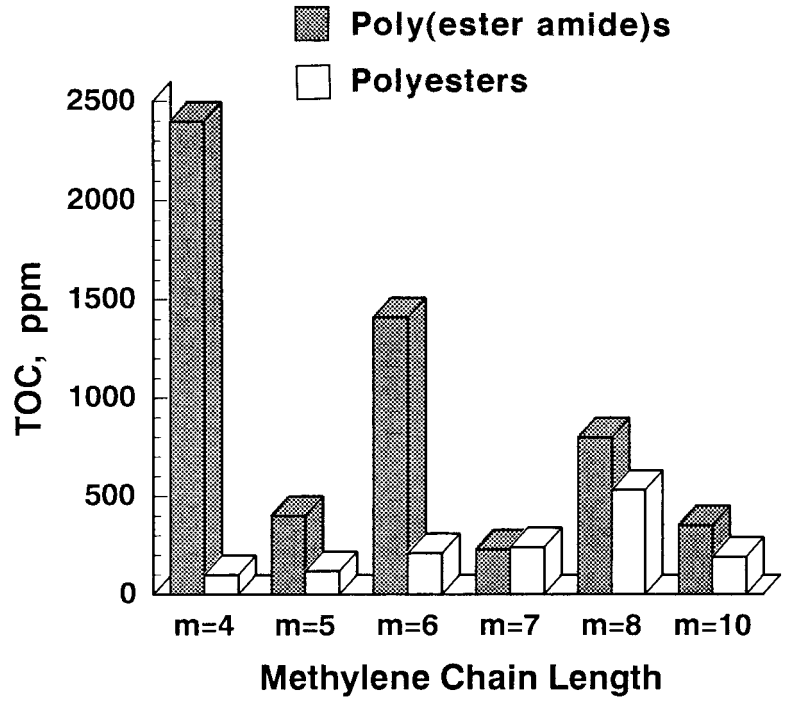


Figure 10 Enzymatic degradation of poly(ester amide)s **7a–7f** and polyesters **4** by papain. Conditions: enzyme, 250 units; sample, 25 mg; phosphate buffer, pH 7.0, 2 mL; incubated at 80 strokes/min at 37°C for 24 h.

solution containing papain (Fig. 10). Above all, it is noteworthy that poly(ester amide) **7a** with the methylene chain length of 4 showed an overwhelmingly higher TOC value than the corresponding polyester **4** (m = 4) with the same methylene chain length. In addition, it is of interest that poly(ester amide)s **7b** and **7d** with the methylene chain length of odd numbers (m = 5 and 7, respectively) are less readily degraded than those with the methylene chain length of even numbers (m = 6 and 8) with papain, although the reason is not clear at present.

Poly(ester amide)s have amide and ester linkages, both of which can be cleaved by enzymatic actions. A question may arise as to which bond is preferentially or selectively cleaved by enzymatic hydrolysis. In this respect, Saotome et al.[13] reported that poly(ester amide) containing phenyl alanine does not undergo amide bond hydrolysis but ester bond hydrolysis by the action of α-chymotripsin. Nagata[18] also found that the degradation of poly(ester amide) containing L-alanine units with proteolytic enzymes such as proteinase K, papain, and α-chymotripsin is not caused by the hydrolysis of the peptide linkage but by hydrolysis of the ester linkages. At present, we have qualitative infrared spectral data suggesting the competitive occurrence of the ester-bond and amide-bond cleavages in the presence of papain. Detailed product analysis of the poly(ester amide)s and some model compounds will be needed to clarify the reaction mechanism of the enzymatic hydrolysis of the present poly(ester amide)s.

In summary, eight different poly(ester amide)s **7a–7h** were synthesized by solution polycondensations of the *p*-toluenesulfonic acid salts of *O*,*O*′-bis(α-aminoacyl)-1,4:3,6-dianhydro-D-glucitol **5a–5c** and bis(*p*-nitrophenyl) esters of aliphatic dicarboxylic acids **6a–6f**. Most of these poly(ester amide)s are amorphous, except **7g** and **7h**, which are semicrystalline, and they are soluble in various polar solvents. Soil burial degradation tests of the films of the poly(ester amide)s, followed by SEM observation, BOD measurements in an activated sludge, and enzymatic degradation test using *Porcine pancreas* lipase and papain, indicated that all these poly(ester amide)s are biodegradable and that their biodegradability depends on the molecular structure. Comparison of the biodegradability of the poly(ester amide)s with that of the corresponding polyesters showed that the poly(ester amide)s were, in general, less readily degraded than the corresponding polyesters having the same aliphatic dicarboxylic acid unit, both in composted soil and in an activated sludge. In the enzymatic degradation, some poly(ester amide)s containing dicarboxylic acid components with shorter methylene chain lengths were degraded more readily than the corresponding polyesters even with *Porcine pancreas* lipase, whereas most of the poly(ester amide)s were degraded more rapidly than the polyesters with papain.

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