Biodegradable Polymers Based on Renewable Resources. IV. Enzymatic Degradation of Polyesters Composed of 1,4:3.6-Dianhydro-D-glucitol and Aliphatic Dicarboxylic Acid Moieties

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ABSTRACT: Enzymatic degradation of a series of polyesters prepared from 1,4:3.6-dianhydro-D-glucitol (**1**) and aliphatic dicarboxylic acids of the methylene chain length ranging from 2 to 10 were examined using seven different enzymes. Enzymatic degradability of these polyesters as estimated by water-soluble total organic carbon (TOC) measurement is dependent on the methylene chain length (m) of the dicarboxylic acid component for most of the enzymes examined. The most remarkable substrate specificity was observed for *Rhizopus delemar* lipase, which degraded polyester derived from **1** and suberic acid (m = 6) most readily. In contrast, degradation by *Porcine liver* esterase was nearly independent of the structure of the polyesters. Enzymatic degradability of the polyesters based on three isomeric 1,4:3.6-dianhydrohexitols and sebacic acid was found to decrease in the order of **1**, 1,4:3.6-dianhydro-D-mannitol (**2**), and 1,4:3.6-dianhydro-L-iditol (**3**). Structural analysis of water-soluble degradation products formed during the enzymatic hydrolysis of polyester **5g** derived from **1** and sebacic acid has shown that the preferential ester cleavage occurs at the O(5) position of 1,4:3.6-dianhydro-D-glucitol moiety in the polymer chain by enzymes including *Porcine pancreas* lipase, *Rhizopus delemar* lipase, and *Pseudomonas sp.* lipase. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 338–346, 2000

Key words: biodegradable polymers; 1,4:3-6,dianhydro-D-glucitol; enzymatic degradation

INTRODUCTION

Environmental issues arising from wasted and undegradable plastics has become increasingly serious and critical all over the world. Recycling of materials and energy recovery are obviously primary strategies for reducing plastic waste, but utilization of biodegradable polymers is also helpful to relieve the difficult situation.[1–5] From another point of view, in concert with the depletion of fossil sources, much attention has been directed towards effective utilization of plant-based biomass resources including wood, agricultural crops and residues, grasses, and components from these sources, as alternative, renewable resources that can be continuously supplied and used for polymers synthesis.[6–8]

Among a wide variety of candidate compounds based on plant-based biomass resources, 1,4:3,6-dianhydrohexitols such as 1,4;3,6-dianhydro-D-

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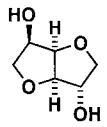
glucitol (**1**), 1,4:3,6-dianhydro-D-mannitol (**2**), and 1,4:3,6-dianhydro-L-iditol (**3**) are promising materials for polymer synthesis. The former two bifunctional monomers are readily available from D-glucose and D-mannose, respectively,[9] and has been used for synthesizing polyesters[10–14] and polyurethanes.[15–17] We have found that polyesters based on these monomers are biodegradable, as judged from degradation in an activated sludge, soil burial degradation, and preliminary enzymatic degradation, as well as spontaneous hydrolytic degradation.[18–22]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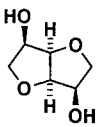
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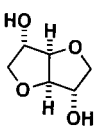
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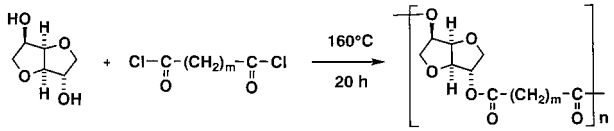
Enzymatic degradation of polymers is often used for rapidly evaluating biodegradability of polymers, although it does not exactly reflect degradation under a natural environment. In a previous article, we have reported a preliminary result that the polyester derived from **1** and sebacic acid undergoes enzymatic degradation by *Rizopus delemar* lipase.[20] As a continuation of this work, we have investigated enzymatic degradation of a series of polyesters (**5**) prepared from 1,4:3,6-dianhydro-D-glucitol (**1**) and eight aliphatic dicarboxylic acids (**4**) of methylene chain lengths ranging from 2 to 10, with seven different enzymes.



1

4a, m = 2
4b, m = 3
4c, m = 4
4d, m = 5
4e, m = 6
4f, m = 7
4g, m = 8
4h, m =10

5a, m = 2
5b, m = 3
5c, m = 4
5d, m = 5
5e, m = 6
5f, m = 7
5g, m = 8
5h, m =10

EXPERIMENTAL

Materials

Commercially available 1,4:3,6-dianhydro-D-glucitol (**1**) and 1,4:3,6-dianhydro-D-mannitol (**2**) were purified by repeated recrystallization from chloroform for **1** and from chloroform-hexane (2 : 1, v/v) for **2.** 1,4:3,6-Dianhydro-L-iditol (**3**) was prepared from **2** via Mitsunobu reaction with benzoic acid in the presence of diethyl azodicarboxylate and triphenylphosphine.[23] The diol was recrystallized from a mixed solvent of *n*-hexane and ethyl acetate (3 : 1, v/v). All the aliphatic dicarboxylic acid dichlorides were prepared by the reaction of the corresponding dicarboxylic acid with thionyl chloride, using a small amount of *N,N*-dimethylformamide as a promoter. These dichlorides were purified by distillation at normal pressure under nitrogen or under reduced pressure.

Polycondensation

Polycondensation of **1** and aliphatic dicarboxylic acid dichloride (**4a–4h**) was carried out in bulk at 160°C, first at normal pressure for 6 h, then under reduced pressure (ca. 25 mmHg) by an aspirator for 2 h, and finally under vacuum (1 mmHg) for 12 h. The resulting polymer was isolated by dissolving the reaction mixture in chloroform and pouring the solution into methanol. It was purified by repeated reprecipitation using chloroform and methanol as a solvent and precipitant pair, and finally dried under reduced pressure to a constant weight.

Characterization

Molecular weights of polyesters (**5a–5h**) were estimated by size-exclusion chromatography (SEC) using chloroform as eluent and standard polystyrene as a reference. ^{1}H- and ^{13}C-NMR spectra of the polyesters were taken by a JEOL JNM-EX-270 operating at 270 MHz (^{1}H) and 67.8 MHz (^{13}C) on solutions in deuteriochloroform using tetramethylsilane as an internal reference. Surfaces of polyester films after soil burial tests were observed with a Hitachi S-4500 scanning electron microscope. Thermal transition temperatures of the polyesters were determined with a Perkin-Elmer DSC-2 differential scanning calorimeter or a Seiko-Electronics DSC100 differential scanning calorimeter 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.

Synthesis of Model Compounds

A sample of 1,4:3,6-dianhydro-2,5-di-*O*-*n*-decanoyl-D-glucitol (3.01 g, 20.5 mmol) was placed in a flask. After it was dissolved in dry pyridine (30 mL) , decanoyl dichloride (7.99 g, 41.9 mmol) was slowly added to the solution with external ice

Table I Characterization Data of Polyesters 5a–5h **Composed of 1,4:3,6-Dianhydro-D-glucitol and Aliphatic Dicarboxylic Acid Units**

Polyester	m[a]	M_n,[b] $\times 10^4$	M_w/M_n[b]	T_g,[c]°C	T_m,[c]°C	H,[c,d] J/g	T_d,°C	$T_{d1/2}$,[e]°C
5a	2	0.77	1.8	36	—	—	330	400
5b	3	2.0	1.7	28	—	—	332	412
5c	4	2.6	1.5	21	—	—	331	410
5d	5	2.2	1.4	11	—	—	330	418
5e	6	2.8	1.4	10	—	—	332	423
5f	7	1.8	1.7	−1	54	22	331	421
5g	8	2.0	1.4	−10	61	24	334	425
5h	10	1.7	1.7	−8	70	24	338	425

[a]Number of methylene units in aliphatic dicarboxylic unit.
[b]Determined by SEC in chloroform (polystyrene standard).
[c]Determined by DSC (heating rate, 10°C/min).
[d]Heat of fusion.
[e]T_d and $T_{d1/2}$ denote initial and half decomposition temperatures, respectively, determined by TGA.

cooling. After the addition, the reaction mixture was stirred at 0–20°C for 1 h and subsequently at 80°C for 2 h. Pyridine was removed by distillation, and the desired product was extracted from the residue with toluene; yield—63.5 g (68%). The structure was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy. 1,4:3,6-Dianhydro-2,5-di-*O*-*n*-decanoyl-D-mannitol was also synthesized in a similar manner.

Enzymatic Degradation

The enzymes used in the present investigation were *Porcine pancreas* lipase (Sigma Chemical), *Porcine liver* esterase (Sigma Chemical), *Rhizopus delemar* lipase (Seikagaku Corp.), *Rhizopus arrhizus* lipase (Sigma Chemical), *Pseudomonas sp.* cholesterol esterase (Wako Pure Chemical), *Pseudomonas sp.* lipase (Wako Pure Chemical), and *Streptomyces rochei* carboxylase (Wako Pure Chemical). All these enzymes were used as supplied. A powdery sample (25 mg) was placed in each test tube and dissolved in a few mL of chloroform. By rotating the test tube and slowly evaporating the solvent, the bottom part of the inner wall of the test tube was coated with a thin film of the polyester (estimated surface area, 11 cm^2). A phosphate buffer solution of pH 7.0 (10 mL) and enzyme (250 units except for *Pseudomonas sp.* cholesterol esterase and *Pseudomonas sp.* lipase (25 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 total organic carbon concentration dissolved in the buffer was 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 an activated sludge taken from a sewage plant in Meito-ku, Nagoya. A sample (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 the complete oxidation of the sample.

RESULTS AND DISCUSSION

Polyesters **5a–5h** were prepared by bulk polycondensations of 1,4:3,6-dianhydro-D-glucitol (**1**) and eight different dicarboxylic acid dichlorides **4a–4h** according to the procedure reported previously.[20] Table I summarizes some of the characterization data of the polyesters used for the enzymatic degradation. Except for polyester **5a** from **1** and succinic dichloride **4a,** molecular weights of all the polyesters were in the range of 1.7×10^4–2.8×10^4. The glass transition temperatures of these samples decrease with increasing methyl-

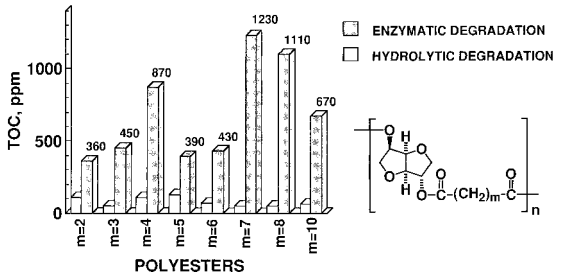


Figure 1 Enzymatic degradation test of polyesters **5a–5h** using *Porcine pancreas* lipase. Polyester, 25 mg; enzyme, 250 units; phosphate buffer (pH 7.0), 2 mL; incubated at 80 strokes per min for 24 h at 37°C.

ene chain length of the dicarboxylic acid units. Polyesters **5f–5h** derived from **1** and azelaic, sebacic, or dodecandioic acids (m = 7, 8, 10) respectively are partially crystalline, and their melting points increase with increasing methylene chain length of the diacid units. The onset temperatures of decomposition did not change very much, reflecting the decomposition of 1,4;3,6-dianhydrdo-D-glucitol units, whereas the half decomposition temperature at which the residual sample weight decreased to 50% of the original sample weight increased slightly with the increase in the methylene chain length of the dicarboxylic acid units.

Enzymatic Degradation of Polyesters

Enzymatic degradation of the polyesters was monitored by TOC measurement of the phosphate buffer solution containing water-soluble degradation products. 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 hydrolysis by subtracting the TOC value in the blank test without enzyme from the observed value with enzyme.

In general, esterases that hydrolyze esters are relatively low in substrate specificity. They catalyze hydrolysis of various types of esters and polyesters. Figure 1 graphically represents the results on the enzymatic degradation of polyesters by *Porcine pancreas* lipase. It is known that *Porcine pancreas* lipase is highly active to polyesters, and is not affected very much by crystallinity.[24] Among the eight different polyesters, polyesters **5c, 5f,** and **5g** derived from adipic (m = 4), azelaic (m = 7) and sebacic (m = 8) acids, respectively, showed higher enzymatic degradability than the other five polyesters. In sharp contrast to this dependence on the methylene chain length, nearly the same TOC values were obtained on all the polyesters, when they were treated with *Porcine liver* esterase (see Table II).

Figure 2 presents the results on the enzymatic degradation of the polyesters by *Rhizopus delemmar* lipase. *Rhizopus delemmar* lipase is an enzyme originating from filamentous fungi, and is affected by the crystallinity of substrates.[25] Clearly, polyester **5e** containing suberic acid (m = 6) showed a specifically high degradability. Polyesters **5f** and **5g** from azelaic (m = 7) and

Table II Enzymatic Degradability of Polyesters 5a–5h based on 1.4:3,6-Dianhydro-D-glucitol and Aliphatic Dicarboxylic Acid (pH, 7.0; temp., 37°C; time, 24 h)

Polyester	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**
m[a]	2	3	4	5	6	7	8	10
Enzyme	TOC[b] ppm							
Porcine pancreas lipase (250 units)	360	450	870	390	430	1230	1100	670
Porcine liver esterase (250 units)	1090	1060	960	960	940	960	970	1050
Rhizopus delemar lipase (250 units)	0	30	50	110	3740	760	1000	230
Rhizopus arrhizus lipase (250 units)	0	50	130	190	1000	350	410	50
Pseudomonas sp. cholesterol esterase (25 units)	50	200	100	1320	1990	1300	1420	830
Pseudomonas sp. lipase (25 units)	50	220	910	2550	2500	1350	1720	750
Streptomyces rochei carboxyesterase (250 units)	0	30	10	0	0	30	0	0

[a]Number of the methylene units in aliphatic dicarboxylic acid unit.
[b]Error limit, +10%.

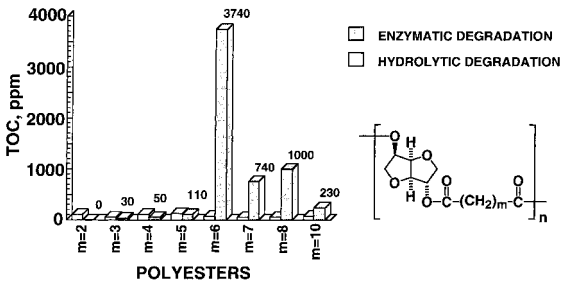


Figure 2 Enzymatic degradation test of polyesters **5a–5h** using *Rhizopus delemar* lipase. Polyester, 25 mg; enzyme, 250 units; phosphate buffer (pH 7.0), 2 mL; incubated at 80 strokes per min for 24 h at 37°C.

sebacic (m = 8) acids, respectively, also showed relatively high degradability. It is likely that the partial crystallinity of these polyesters is responsible for lowering their enzymatic degradability to some extent. In contrast, polyesters containing aliphatic diacids with a methylene chain length of five or shorter were hardly or only slightly degraded by this enzyme. The observation may be indicative of the substrate specificity of this enzyme. *Rhizopus arrhizus* lipase, also originating from filamentous fungi, acts as an enzyme decomposing polyesters.[26] When the polyesters were in contact with this enzyme, a similar, but less specific, trend was observed in the TOC values of the polyesters, i.e., polyester **5e** containing suberic acid (m = 6) showed the highest TOC value followed by polyesters **5f** and **5g** from azelaic (m = 7) and sebacic (m = 8) acids (see Table II).

Figure 3 shows the results on the enzymatic degradation tests of the polyesters by *Pseudomonas sp.* cholesterol esterase from bacteria. Polyesters containing aliphatic dicarboxylic acid units with a methylene chain length of 5 or longer were relatively easily degraded with this enzyme. *Pseudomonas sp.* lipase showed a hydrolysis activity to the polyesters containing dicarboxylic acid units with a methylene chain length of 4 or longer, particularly to polyesters **5d** and **5e** derived from pimelic acid (m = 5) and suberic acid (m = 6) (see Table II). Enzymatic degradation by either *Pseudomonas sp.* cholesterol esterase or *Pseudomonas sp.* lipase appears to be retarded to some extent for the partially crystalline polyesters **5f–5h.**

Streptmyces rochei carboxyesterase was found to be hardly effective for the enzymatic degradation of all the polyesters examined in the present investigation (see Table II), although the enzyme was somewhat effective for the enzymatic degradation of furan-containing polyesters.[27] Table II summarizes all the enzymatic degradation tests on polyesters **5a–5h** for comparison.

The effects of chemical structure of aliphatic polyesters on the enzymatic hydrolysis have been investigated by several groups. For example, Fields et al. examined enzymatic hydrolysis of a series of polyesters prepared from combinations of C_2–C_{12} aliphatic dicarboxylic acids and C_4–C_{12} α,β-alkanediols, and concluded that the aliphatic polyesters most rapidly hydrolyzed were those containing six carbon atoms between ester linkages.[28] Diamond et al. also reported that the enzymatic degradability of aliphatic polyesters was related to the carbon chain spacing between ester groups, and polyesters having a longer methylene chain were degraded only slightly.[29]

Needless to say, not only primary structure of polyesters but also ordered structure are the important factors affecting enzymatic degradability of polyesters. Huang and his coworkers showed that the biodegradation of semicrystalline poly(ε-caprolactone) proceeded more preferentially in an amorphous region than in a crystalline region.[30] Mochizuki et al. reported that the enzymatic degradation of poly(ε-caprolactone) fibers of different draw ratios decreased with an increase in draw ratio.[31] In the enzymatic hydrolysis of poly(butylene succinate-*co*-ethylene succinate)s, they also suggested that the degree of crystallinity may be the dominant factor influencing the rate of enzymatic degradation.[32]

In general, esterases that hydrolyze polyesters are known to be relatively low in substrate specificity. Actually, all the enzymes except one used in the present experiments hydrolyzed polyesters **5a–5h** to a greater or less extent. Because these polyesters contain rigid and bulky 1,4:3,6-dianhy-

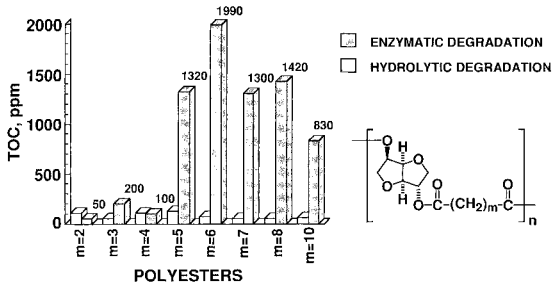


Figure 3 Enzymatic degradation test of polyesters **5a–5h** using *Pseudomonas sp* . cholesterol lipase. Polyester, 25 mg; enzyme, 25 units; phosphate buffer (pH 7.0), 2 mL; incubated at 80 strokes per min for 24 h at 37°C.

Table III Biodegradability of Polyesters 5a–5h Evaluated by BOD Measurement in an Activated Sludge at 25°C

Polyester	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**
m[a]	2	3	4	5	6	7	8	10
Days	BOD-Based Biodegradability, %							
14	0.8	5.1	19.5	17.3	29.6	2.7	25.1	7.6
21	1.9	7.1	23.8	33.0	51.8	9.0	33.7	20.8
28	2.7	8.7	26.6	47.3	61.6	29.4	40.1	35.1
35	4.3	9.1	29.5	60.6	68.2	42.1	43.5	45.1
42	5.6	9.7	31.6	66.2	68.8	53.0	45.1	57.5

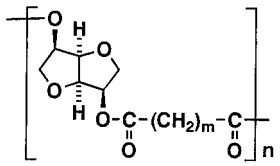
[a]Number of the methylene units in the aliphatic dicarboxylic unit of the polyester.

dro-D-glucitol units as the diol component, a relatively wide spacing between the ester linkages would be required for the attack of enzymes. The observed trend that five out of the seven enzymes examined showed a maximum in hydrolytic activity between polyesters **5e** and **5g** of a medium methylene chain length (m = 5 and 8) is at least partly related to the hydrophilic-hydrophobic balance within the polymer chain. In addition, polyesters **5f–5h** are partially crystalline, and as a consequence, their enzymatic degradation should be suppressed to some extent. It is therefore understandable that in most cases, polyester **5e** exhibited a higher enzymatic degradability than the other polyesters. The diacid methylene length independence of the hydrolytic activity of *Porcine liver* esterase is very unique, which may suggest a different enzymatic degradation mechanism.

For comparison with the enzymatic degradability, BOD measurements on the polyesters were carried out in an activated sludge according to JIS K 6950. The results are summarized in Table III. The BOD-based biodegradability is defined in the Experimental section. Similar BOD tests on 1,4:3,6-dianhydro-D-glucitol and suberic acid themselves revealed that both of these monomers showed the BOD-based biodegradability of 70–80% after 28 days. Polyester **5e** from suberic acid (m = 6) showed the fastest degradation, and the BOD-based biodegradability reached as high as 62% after 28 days. Polyester **5d** from pimelic acid (m = 5) showed the BOD-based biodegradability of 66% after 42 days. In contrast, the BOD-based biodegradability of polyesters **5a** and **5b** from succinic acid (m = 2) and glutaric acid (m = 3) was below 10%. This is consistent with the low degradability observed in their soil burial degradation.[20] Polyester **5h** from dodecandioic acid (m = 10) showed a relatively high BOD-based biodegradability in an activated sludge, although the polyester was only slowly degraded in the soil burial test. This discrepancy is not unreasonable, because micro-organisms growing in an activated sludge are different from those in soil. In general, there are more bacteria than filamentous fungi in an activated sludge, and hence, degradation of polyester **5g** based on **1** and sebasic acid (m = 8), which was most rapidly degraded by filamentous fungi in soil burial,[20] seems to have been retarded in the activated sludge.

Effect of Configuration of Diol Components

Effect of diol components on the enzymatic degradation of polyesters was investigated on three different polyesters, **5g, 6,** and **7,** composed of 1,4:3,6-dianhydro-D-glucitol (**1**), 1,4:3,6-dianhydro-D-mannitol (**2**), or 1,4:3,6-dianhydro-L-iditol (**3**), respectively, as a diol component and sebacic acid as a common diacid component. The characterization data of these polyesters and the results on the enzymatic degradation using six different enzymes are listed in Table IV.



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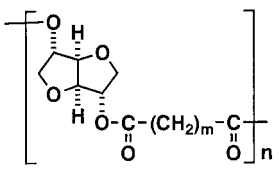

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Among these enzymes, *Porcine liver* esterase degraded the three stereoisomeric polyesters to a similar extent irrespective of the diol components. All the other enzymes degraded polyester **5g**

Table IV Enzymatic Degradability of Polyesters Based on 1,4:3,6-Dianhydrohexitol and Sebacic Acid Moieties[a]

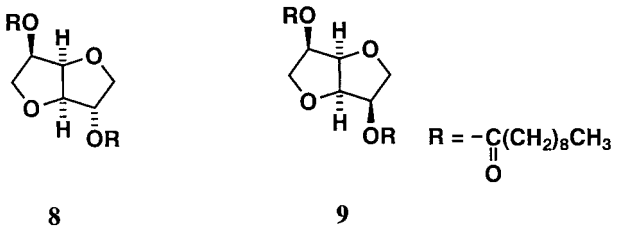
Polyester	**5g**	**6**	**7**
M_n	1.3×10^4	1.3×10^4	2.9×10^4
T_m, °C	51	—	140
T_g, °C	−5	−8	0
$T_{d5\%}$[b], °C	377	395	387
ΔH[c], mJ/mg	26	—	44

Enzyme	TOC, ppm		
Porcine pancrease lipase	880	470	450
Porcine liver esterase	930	960	1140
Rhizopus delemar lipase	1040	20	0
Rhizopus arrhizus lipase	300	0	0
Pseudomonas sp. cholesterol esterase[d]	1100	30	40
Pseudomonas sp. lipase[d]	1310	170	0

[a]Temp., 37°C; time 24 h; polyester 25 mg; enzyme, 250 units; phosphate buffer (pH 7.0), 2 mL.
[b]$T_{d5\%}$ denotes decomposition temperature of 5% weight loss determined by TGA.
[c]Heat of fusion determined by DSC.
[d]Enzyme, 25 units.

faster than polyesters **6** and **7.** In polyester **6,** both of the carbon-oxygen bonds connecting a 1,4; 3,6-dianhydro-D-mannitol moiety with ester linkages in the polymer chain are in the *endo*-position, thus presumably making the access of enzymes unfavorable due to steric hindrance. In polyester **7,** both of the carbon-oxygen bonds connecting 1,4:3,6-dianhydro-L-iditol moiety with ester linkages are in the *exo*-position, and therefore, the approach of enzyme appears to be favorable. Nevertheless, polyester **7** was found to be most reluctantly degraded among the three polyesters. The lower sensitivity of polymer **7** to enzymatic degradation seems to be ascribable to its higher crystallinity.

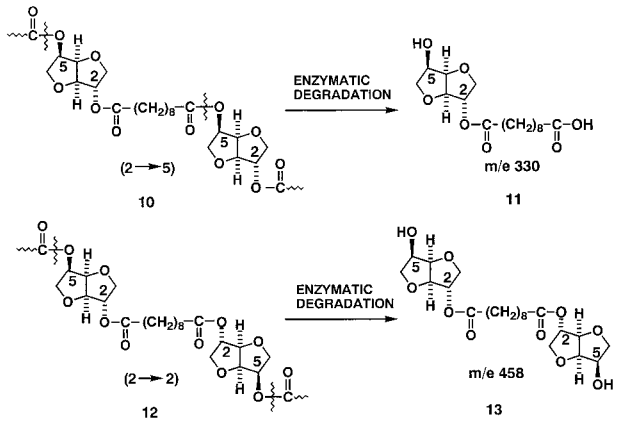
To clarify the geometrical structural effect on the enzymatic degradation, 1,4:3,6-dianhydro-2,5-di-*O*-*n*-decanoyl-D-glucitol (**8**) and 1,4:3,6-dianhydro-2,5-di-*O*-*n*-decanoyl-D-mannitol (**9**) were prepared as model compounds for polyesters **5g** and **6,** respectively, and their degradation by *Porcine pancreas* lipase was compared. The TOC value for **8** under the conditions similar to those for the enzymatic degradation of the polyesters was 550 ppm, much higher than that for **9** (90 ppm). The higher enzymatic degradability of **8** is consistent with the higher enzymatic degradability of polyester **5g** compared with that of polyester **6.**



8 **9**

Analysis of Enzymatic Degradation Products

Analysis of degradtion products in enzymatic hydrolysis is essential for the clarification of the enzymatic degradation mechanism. Polyester **5g** composed of 1,4:3,6-dianhydro-D-glucitol and sebacic acid units was enzymatically hydrolyzed by *Porcine pancreas* lipase, and the water-soluble products were examined by SEC, NMR, and MS. The SEC analysis showed that there were two compounds whose elution volumes were 16.8 and 17.8 mL, respectively. These compounds were isolated and identified as a monoester **11** and a diester **13** (Scheme 1) by ^{1}H- and ^{13}C-NMR, together with mass spectroscopy. It is noteworthy that in both of these compounds, ester linkages remained at the O(2)-position of the 1,4;3,6-dianhydro-D-glucitol units, whereas no ester linkage remained at the O(5)-position. As illustrated in Scheme 1, **11** is produced by the regiospecific ester cleavage at the O(5) position of a (2 → 5)-linked or (5 → 2)-linked dianhydroglucitol (G)-sebacic acid (S)-dianhydroglucitol (G) triad unit **10** in the polymer chain, and **13** is formed by the



Scheme 1 Regiospecific ester cleavage in the enzymatic hydrolysis of polyester **5g** derived from 1,4:3,6-dianhydro-D-glucitol (**1**) and sebacic acid.

regioselective ester cleavage at the O(5) position of a (2 → 2)-linked GSG unit **12**. Polycondensation of **1** and sebacic acid was found to take place randomly to give a statistical polyester containing (2 → 2), (2 → 5 and 5 → 2)-, and (5 → 5) linked SGS units in the ratio of nearly 1 : 2 : 1.[19] The molar ratio of the monoester **11** to diester **13** in the enzymatic hydrolysis was found to be roughly 2 : 1. Therefore, the observation above is strongly indicative of preferential cleavage of the ester linkage at the O(5)-position in the enzymatic hydrolysis of the polyester by *Porcine pancreas* lipase. Similar results were obtained with *Rhizopus delemar* lipase and *Pseudomonas sp.* lipase.

It seems reasonable to assume that the regiospecific cleavage arises from the fitness between the geometrical structure around the ester linkage and the steric requirement of the binding site of the enzyme. However, in view of the fact that the regioselective cleavage occurred for the plural number of enzymes, the geometrical structure around the ester linkage may be the decisive factor. Similar regiospecificity was also found in the enzymatic hydrolysis of the aforementioned model compound, 1,4:3,6-dianhydro-2,5-di-*O*-*n*-decanoyl-D-glucitol (**8**): the enzymatic hydrolysis of **8** by the action of *Porcine pancreas* lipase gave exclusively 1,4:3,6-dianhydro-2-*O*-*n*-decanoyl-D-glucitol having an ester linkage at the O(2)-position, in agreement with the observation in the enzymatic hydrolysis of the polyester described above under the identical conditions.

In summary, enzymatic degradability of the polyesters based on **1** and aliphatic dicarboxylic acids is more or less dependent on the methylene chain length (m) of the dicarboxylic acid component for most of the seven different enzymes examined. As a whole, polyester **5e** composed of **1** and suberic acid (m = 6) showed the highest enzymatic degradability. Although it is difficult to interpret the observed structural dependence of the enzymatic degradability in terms of a single factor, a relatively large spacing between the ester linkages and suitable hydrophilic-hydrophobic balance of the polyesters are important factors for the facile approach of the enzyme to the substrate and for the effective enzymatic action. Needless to say, the degree of crystallinity also influences the degradability. The results reported herein along with the previously reported degradation in an activated sludge and soil burial degradation of the polyesters[20] suggest that biodegradable polyesters are produced from 1,4:3,6-dianhydro-D-glucitol (**1**) as one of the components, and that their biodegradability can be widely modified by proper molecular and material design.

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