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Biodegradability of Poly(ester-ether) and Poly(ester) Obtained from a Radical Ring-Opening Polymerization of Cyclic Ketene Acetals

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2-Methylene-1,3,6-trioxocane (MTC) and 2-methylene-1,3-dioxepane (MD) were synthesized and polymerized via ring-opening in the presence of a radical initiator. The obtained poly(ester-ether) (PMTC) and poly(ester) (PMD) were found to be enzymatically degradable by total organic carbon (TOC) analysis using lipase. The enzymatic degradability of PMTC was higher than that of PMD. PMTC and PMD were also found to be biodegradable with a biological oxygen demand (BOD)-tester using soil. The degradability of PMTC using soil was also higher than that of PMD. The higher degradability of PMTC by enzyme and soil are thought to be due to its higher hydrophilicity.

Keywords cyclic ketene acetals, poly(ester-ether), poly(ester), radical ring-opening polymerization, biodegradation

Introduction

Aliphatic polyesters have been attracting considerable attention because of their biodegradability. Poly(β-hydroxyalkanoate) is synthesized by many bacteria (1–3). Many synthetic polyesters are hydrolyzed by lipase (4, 5). Several studies on the isolation of microorganisms that degrade poly(L-lactide) have been published recently (6–10). On the other hand, it was reported that poly(ester-ether)s such as poly(1,4-dioxane-2-one) were biodegradable (11, 12). 1,5-Dioxepane-2-one has been polymerized via ring-opening to obtain poly(ester-ether)s constituted of two alternating species, corresponding to β-propiolactone and ethylene oxide (13).

We reported that 2-methylene-1,3,6-trioxocane (MTC) was polymerized via ring-opening in the presence of a radical initiator to obtain a poly(ester-ether) (PMTC) constituted of two alternating species, relating to γ-butyrolactone and ethylene oxide (Scheme 1), and obtained poly(ester-ether) degraded by enzyme (14). MTC could also

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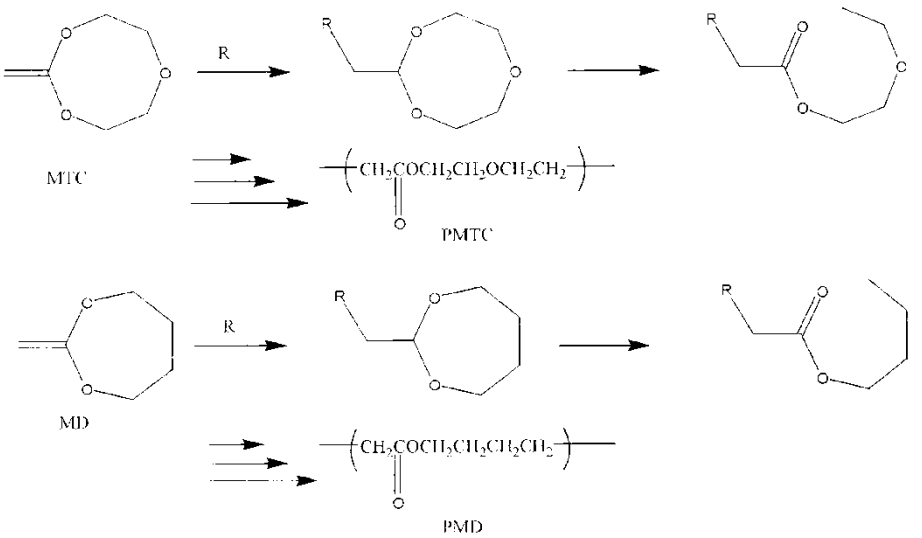

Scheme 1. Ring-opening polymerization of MTC and MD.

be copolymerized with vinyl monomers and the obtained copolymers had enzymatic degradability (15–19). 2-Methylene-1,3-dioxepane (MD) was also polymerized via ring-opening in the presence of radical initiator to obtain polyester (PMD) constituted of polycaprolactone (Scheme 1) (20).

However, only measurement of the water-soluble total organic carbon (TOC) when an enzyme acted on PMTC obtained from cyclic ketene acetal was reported (14), and not the biodegradability of PMD.

We report here on our comparison of degradabilities of poly(ester-ether) (PMTC) obtained from MTC and polyester (PMD) obtained from MD as determined by both two methods, total organic carbon TOC analysis when lipase acted on them and BOD testing using the oxygen consumption method.

Experimental

Materials

MTC was prepared by the previous method (14). MD was prepared from dimethylchloroacetal and 1,4-butanediol by the same method as MTC. Chlorobenzene (CB) was purified by distillation over calcium hydride before use. 2,2′-Azobisisobutyronitrile (AIBN) and di-*tert*-butyl peroxide (DTBP) were used without further purification. Poly(D-3-hydroxybutyrate) (PHB) with a number-average molecular weight (Mn) of 2.1×10^5 was obtained from Mitsubishi Gas Chemical.

Polymerization. The polymerization of MTC was carried out as follows: in a 10 mL sealed polymerization tube, a mixture containing MTC (5.00 g, 3.84×10^{-2} mol) and AIBN (0.126 g, 7.69×10^{-4} mol) was maintained at 60°C for 24 h. The resulting product was precipitated in n-hexane. The precipitated material was dried under reduced pressure to give 4.50 g of a liquid polymer (PMTC) (88%). The average molecular weight of the polymer was determined by gel permeation chromatography (GPC) with a refractive

index detector (Tosoh HLC-8020 GPC). A combined column, TSKgel multi-pore HxL-M, was used with a mobile phase of chloroform at a flow rate of 0.8 mL/min. Polystyrene was used as a molecular-weight standard. Measurement of the glass transition temperature (Tg) of the polymers was carried out using a DSC (Seiko Instruments DSC-220C) at the heating rate of 10°C/min. IR (neat): 2951, 2872, 1736, 1254, 1177, 1127 cm^{-1}. ^1H NMR ($CDCL_3$, 270 MHz): δ 1.72–2.36 (m, 2H, OCH_2CH_2), 2.45 (t, 2H, CH_2COO), 3.42–3.81 (m, 4H, CH_2OCH_2), 4.22 (t, 2H, $COOCH_2$), Mn = 4.7×10^3, Mw/Mn = 10. The polymerization of MTC in CB, the bulk polymerization of MD, and polymerization in CB of MD were carried out the same manner. The details of polymerization of MTC and MD are shown in Table 1.

Biodegradation Test of PMTC and PMD using Lipase by Tortal Organic Carbon (TOC). The enzymatic degradability of the polymers was examined by the rate of solubilization when lipase acted on them. The enzymatic degradability of the polymers was evaluated by TOC analysis when lipase acted on them. A control experiment without enzyme was carried out simultaneously. Films of PMTC and PMD (20 mg) were made from chloroform solutions in test tubes at a height of 15 mm from the bottom of the test tubes. 0.2 mL of 0.2 M phosphate buffer (pH 7.0), 0.2 mL of *Rhizopus arrhizus* lipase in 6 mL of 0.02 M phodphate buffer, and 0.6 mL of pure water were added to each test tube in a total volume of 1 mL. The reaction mixture was shaken reciprocally at 30°C for 24 h. After shaking, the TOC concentration in the filtrate of the reaction mixture was measured with a Shimadzu TOC-5000 analyzer.

Biochemical Oxygen Demand (BOD) Test. BOD was determined with a BOD tester (OM 8001A; Ohkura Denki Co., Tokyo, Japan) by the oxygen consumption method and basically according to ISO 14851 (JIS K 6950) at 25°C using a soil freshly obtained from a forest near Tsukuba City. The incubation medium contained the following (mg/L): K_2HPO_4, 217.5; KH_2PO_4, 85.0; Na_2HPO_4, 260.5; NH_4Cl, 25.0; $CaCl_2 \cdot 2H_2O$, 36.4; $mgSO_4 \cdot 7H_2O$, 22.5; $FeCl_3 \cdot 6H_2O$, 0.25 (pH = 7.4). The concentration of the polymer in the incubation medium was 100 mg/L.

Hydrolysis Test in Phosphate Buffer on PMTC. Hydrolysis of PMTC (60°C, bulk) in phosphate buffer was evaluated using a TOC analyzer and GPC. A film of PMTC (20 mg) was made from chloroform solution in a test tube at the height of 15 mm from

Table 1
Polymerization of MTC and MD

Monomer	Temperature (°C)	Time (h)	Solvent (eq.)	Yield[a] (%)	Mn[b]	Mw/Mn	Tg[c] (°C)
MTC	120	48	CB (1)	77	2700	25	−48.7
MTC	60	24	—	88	4700	10	−47.8
MD	120	48	CB (1)	67	2400	23	−52.6
MD	60	24	—	87	7500	4	−55.8

[a]Insoluble part in n-hexane.
[b]Based on polystyrene.
[c]at the heating rate of 10°C/min.

the bottom of the test tube. 0.2 mL of 0.2 M phosphate buffer (ph 7.0), 0.2 mL of 0.02 M phosphate buffer (ph 7.0), and 0.6 mL of pure water were added to the test tube in a total volume of 1 mL. The reaction mixture was shaken reciprocally at 30°C for 1 week. After the shaking, TOC and GPC of the filtrate were measured with a TOC analyzer and GPC.

Results and Discussion

MTC and MD were ring-opening polymerized in the presence of radical initiator such as AIBN or DTBP. Glass transition temperature (Tg) of PMTC was higher than that of PMD. Because oxygen atom is included in the backbone of PMTC, the hydrogen bond of PMTC might contribute to higher Tg. PMTC and PMD were hydrolyzed by *Rhizopus arrhizus* lipase. The results are shown in Table 2. The solubilization percentage of PMTC was calculated according to Equation (1). The value when PMTC degraded completely is:

$$20000\ (\mathrm{mg/L}) \times 12 \times 6/130 = 11077\ (\mathrm{ppm}) \tag{1a}$$

6 carbons are contained in 1 unit of PMTC. The molecular weight of MTC is 130. Experimental data was:

$$9560 - 950 - 22 = 9440\ (\mathrm{ppm}) \tag{1b}$$

The solubilization percentage of PMTC was:

$$9440/11077 \times 100 = 85\ (\%) \tag{1c}$$

The solubilization percentage of PMTC was 85% and 47%. The solubilization percentage of PMD was 9% and 16%. Although PMTC and PMD could be hydrolyzed by lipase, PMTC could be hydrolyzed to a higher degree than PMD. In the substrate control, degradation of PMTC was higher than that of PMD. This indicates that the hydrophilicity of PMTC is higher than that of PMD. Therefore, dispersibility of PMTC in water became higher than that of PMD and more ester groups in PMTC attacked by enzyme exposed to the enzyme.

PMTC and PMD were degraded by microorganisms in soil. Figure 1 shows the theoretical oxygen demand (TOD) values.

Table 2
Hydrolysis of polymer by lipase[a]

	TOC (ppm)			
	PMTC (120°C in CB)	PMTC (60°C in bulk)	PMD (120°C in CB)	PMD (60°C in bulk)
Sample	9560	6230	1640	2570
Substrate control	950	1010	420	490

[a]Enzymatic control 22 ppm.

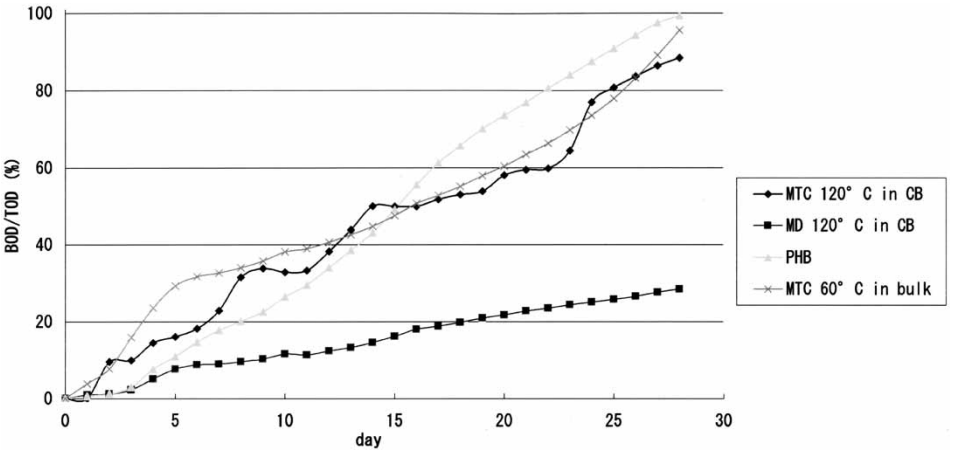

Figure 1. Biodegradability of PMTC and PMD by BOD-tester using soil.

BOD/TOD value in PMTC at 28 days was calculated according to Equation (2).

$$\left(\text{CH}_2\text{COCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\right) + 7\text{O}_2 \longrightarrow 6\text{CO}_2 + 5\text{H}_2\text{O} \qquad (2a)$$

Because the weight of PMTC was 30 (mg), molar value of PMTC was $30/130 = 2.31 \times 10^{-4}$ mol. So, when PMTC degraded completely,

$$2.31 \times 10^{-4} \times 7 \times 32 \times 1000\ (\text{mL})/300\ (\text{mL}) = 172\ (\text{ppm}) \qquad (2b)$$

Because BOD was 152 ppm at 28 (days), BOD/TOD was calculated as follows:

$$152/172 \times 100 = 88\ (\%) \qquad (2c)$$

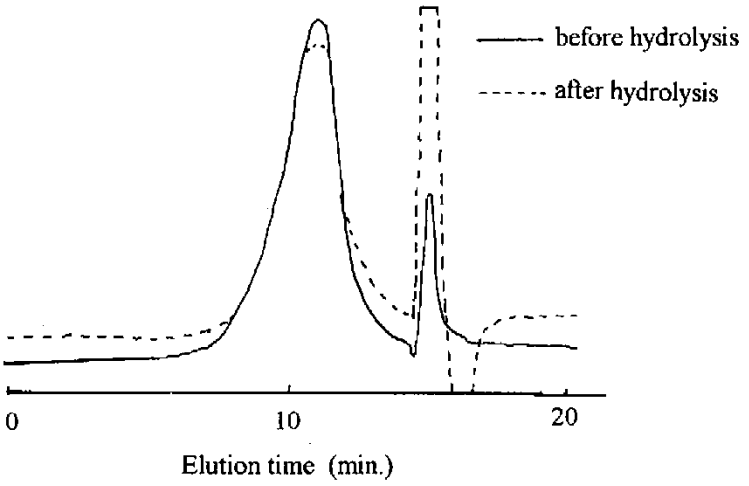

Figure 2. GPC pattern of before and after hydrolysis of PMTC in phosphate buffer.

BOD/TOD values of PMTC (in CB, 120°C, and bulk, 60°C at 28 (days)) were 88% and 95%. Those of PMD (in CB, 120°C and PHB were 28% and 99%. BOD/TOD value is also higher in PMTC than that in PMD. The dispersibility of PMTC in water might be superior to that of PMD in a BOD tester. The results of enzymatic degradability and degradability in soil of PMTC and PMD, indicates that PMTC is degraded more easily than PMD. The fact that PMTC is degraded similarly to PHB, means that PMTC is a polymer that can be degraded easily.

The TOC results in a hydrolysis test of PMTC in phosphate buffer was 910 ppm. This result is consistent with the result for the substrate control of PMTC shown in Table 2. The GPC pattern before and after hydrolysis test in phosphate buffer was substantially consistent as shown in Figure 2. However, oligomer of PMTC increased after the hydrolysis. This indicates that PMTC was hydrolyzed slightly by a phosphate buffer. These results of hydrolysis of PMTC by lipase and phosphate buffer indicate that PMTC is hydrolyzed by lipase and hardly hydrolyzed by phosphate buffer.

Conclusion

The biodegradabilities of PMTC and PMD were ascertained using a TOC-analyzer and a BOD-tester. The degradability of PMTC was found to be higher than that of PMD. The higher degradability of PMTC was attributable to higher hydrophilicity.

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