Synthesis and Enzymatic Degradation of High Molecular Weight Aliphatic Polyesters

HIROYUKI SHIRAHAMA, YOSHIHIDE KAWAGUCHI, MOHAD S. ALUDIN, HAJIME YASUDA

Hiroshima University, Faculty of Engineering, Department of Applied Chemistry, Higashi-Hiroshima 739-8527, Japan

Received 1 February 2000; accepted 3 April 2000

ABSTRACT: The aliphatic polyesters with high molecular weight have been prepared according to two methods. First is the synthesis of the polyesters by polycondensation of dimethyl succinate (DMS) with 1,4-butanediol (BD) using various metal alkoxides as a catalyst. Among the metal alkoxides used, titanium tetraisopropoxide [Ti(O*i*Pr)$_4$] gave the best results (highest molecular weight and yield). Thus, we have prepared aliphatic polyesters using a variety combinations of diesters [MeOOC—(CH$_2$)$_x$—COOMe, x = 2–8] with BD by the catalysis of Ti(O*i*Pr)$_4$. The polyesters with high number-average molecular weight (M_n > 35,000), except dimethyl adipate (DMA, x = 4)/BD polyester (M_n = 26,900), were obtained in high yield. The melting temperatures (T_m) of polyesters were relatively low (43.4–66.8°C) except that (115.6°C) of the DMS/BD polyester. Second is the synthesis of high molecular weight polyesters by chain extension reaction of lower molecular weight (M_n = 15,900–26,000) polyesters using hexamethylene diisocyanate (HDI) as a chain extender. The M_n values of chain-extended polyesters consequently increased more than two times (M_n = 34,700–56,000). The thermal properties of polyesters hardly changed before and after chain extension. Enzymatic degradations of the polyesters were performed using three different enzymes (cholesterol esterase, lipase B, and *Rhizopus delemar* lipase) before chain extension. The enzymatic degradability varied depending on both thermal properties of polyesters [melting temperature and heat of fusion (crystallinity)] and the substrate specificity of enzymes, but it was the following order: cholesterol esterase > lipase B > *R. delemar* lipase. The ^{1}H-NMR spectrum of water-soluble degraded products of the polyester indicated that the polyester was degraded into a condensation product of diol with diester in a monomer form. The enzymatic degradation of chain extended polyesters was slightly smaller than that before chain extension, but proceeded steadily. J Appl Polym Sci 80: 340–347, 2001

Key words: high molecular weight aliphatic polyester; polycondensation; chain extension; enzymatic degradation

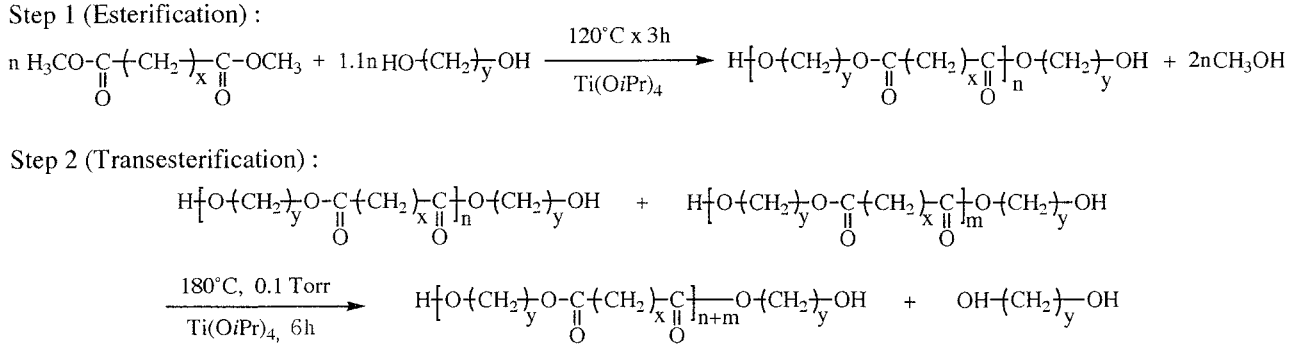
INTRODUCTION

From an environmental perspective, biodegradable polymers produce an attractive alternative to conventional nonbiodegradable plastics. Not much research has been done on the syntheses, physicochemical properties, and degradations of biodegradable polymers over the past few decades. Among those polymers, one of the successfully developed polymers is aliphatic polyester, due to its suitable biodegradability and processability. However, aliphatic polyesters with high number-average molecular weight (M_n > 30,000) still cannot be obtained easily by polycondensation of dicarboxylic acids (or

Correspondence to: H. Shirahama, H. Yasuda.

Contract grant sponsor: Grant-in-aid for Scientific Research, Ministry of Education, Science, Sports, and Education (Japan); contract grant number: 11217211.

Journal of Applied Polymer Science, Vol. 80, 340–347 (2001)



Scheme 1

diesters) with diols. To achieve high molecular weight polymers, several catalysts such as the Al–porphyrin complex,[1] $ZnEt_2$,[2] and lipase[3] were used, but the molecular weights of polyesters obtained were still not very high (M_n < ca. 15,000).

For obtaining high molecular weight polyesters by polycondensation, we have adopted two methodologies; i.e., one is the use of transition metal alkoxides having transesterification ability, and the other is the chain extension reaction of lower molecular weight polyesters (see Schemes 1 and 2). In the present study, we report the synthesis of high molecular weight aliphatic polyesters by polycondensation of diesters with diols with and without chain extension reaction, and the enzymatic degradation of those polyesters.

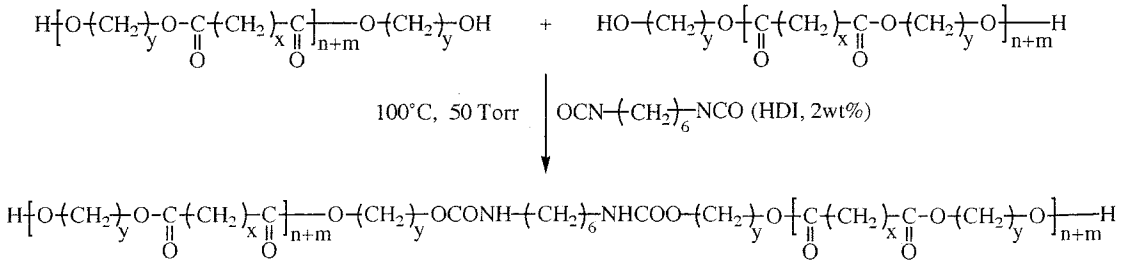
EXPERIMENTAL

Materials

The diesters used [MeOOC—$(CH_2)_x$—COOMe, x = 2–8] were dimethyl succinate (DMS), dimethyl glutalate (DMG), dimethyl adipate (DMA), dimethyl pimelate (DMP), dimethyl suberate (DMSb), dimethyl azelate (DMAz), and dimethyl sebacate (DMSe). All these diesters were dried over CaH_2 overnight, distilled twice under reduced pressure, and kept on activated molecular sieves (4 Å) just before use. A variety of diols [HO—$(CH_2)_y$—OH], y = 2–7] were also used. However, the preliminary experiments and our previous studies[4,5] revealed that 1,4-butanediol (BD) gave the highest molecular weight and the highest yield. Hence, in this work we used BD as diol, which was dried over Na metal overnight and distilled twice under reduced pressure. Metal (III–V) alkoxides used as polycondensation catalyst were $Sm(OiPr)_3$, $Ti(OEt)_4$, $Ti(OiPr)_4$, $Zr(OBu)_4$, $Ge(OEt)_4$, $Nb(OEt)_5$, and $Ta(OEt)_5$. These metal alkoxides and hexamethylene diisocyanate (HDI) as chain extender were used as received. Tolylene 2,4-diisocyanate (TDI) is a typical chain extender, but the polyesters and polyurethanes containing an aromatic unit such as TDI are known to be hardly biodegraded.[6,7] Thus, we used HDI as a chain extender in this work.

Preparation of Polyesters

All operations for polymer synthesis were conducted using conventional Schlenk techniques under an argon atmosphere. Scheme 1 shows the schematic representation of polycondensation of diesters with diols. The polycondensation was



Scheme 2

Table I Polycondensation of DMS with BD by Metal Alkoxides[a]

Metal Alkoxide (Catalyst)	Yield (%)	$M_n/10^4$ [b]	M_w/M_n [b]
$Ti(OEt)_4$	82.5	1.62	1.32
$Ti(OiPr)_4$	87.0	6.10	1.70
$Zr(OBu)_4$	63.0	1.05	1.55
$Nb(OEt)_5$	85.0	2.03	1.62
$Ta(OEt)_5$	71.7	1.14	1.73

[a] Conditions are the same as stated in the Experimental section.
[b] Determined by GPC.

carried out in bulk with stirring in an oil bath using the following optimum conditions.[5] DMS (5.22 mL, 40 mmol) and BD (3.90 mL, 44 mmol) (BD/DMS = 1.1 in molar ratio) were placed in a side-arm Schlenk tube, then $Ti(OiPr)_4$ (0.052 mL, 0.2 mol %/monomer) was added into the tube. The tube was immersed in an oil bath thermostated at 120°C for ca. 3 h until the by-product (methanol) was completely distilled out (step 1: esterification in Scheme 1). Subsequently, the reaction mixture was gradually evacuated to ca. 0.1 Torr and heated to 180°C for another 6 h (step 2: transesterification). The resulting crude product was dissolved in chloroform, and was poured into methanol for precipitating the polymer. The precipitate was washed with methanol twice and with diethylether. The polyester thus obtained was dried *in vacuo* for 3 days at the ambient temperature.

Chain Extension Reaction

Aliphatic polyesters with lower molecular weight were prepared by changing some conditions in polycondensation (molar ratio of diol to diester 1.1→1.2, vacuum pressure 0.1→1.0 Torr). Because a small excess of the diol over the diester (molar ratio 1.2) was used, one of two-end groups of the obtained polyester should be a hydroxyl group (—OH). Therefore, the chain extension reaction between hydroxyl and isocyanate groups occurs according to Scheme 2. Concretely, the chain extension reaction was carried out as follows. The polyester (1.0 g) was placed in a Schlenk tube containing the solvent (chloroform 8 mL), and was dissolved with stirring. Then, hexamethylene diisocyanate (HDI 0.2 mL, ca. 2 wt %/polyester) was added into the tube. The mixture was heated to 100°C in an oil bath, and the solvent was completely trapped under reduced pressure (ca. 50 Torr) for 20 min. The reaction was allowed to continue for another 1 h. The purification of chain extended polyester was conducted in a similar manner described above.

Characterization of Polyesters

The number-average molecular weight (M_n) and the polydispersity (M_w/M_n) of polyesters were determined by gel permeation chromatography (GPC) at column oven temperature 40°C on a Tosoh GPC system equipped with four TSK gel columns ($G2000H_{HR}$ + $G3000H_{HR}$ + $G4000H_{HR}$ + $G5000H_{HR}$) using a differential refractometer, calibrating with standard polystyrenes. Chloroform was used as an eluent at the flow rate of 1.0 mL/min. The thermal properties of polyesters, glass transition temperature (T_g), melting temperature (T_m), and heat of fusion (ΔH_m), were measured by differential scanning calorimetry (DSC) on a Seiko SSC5100 DSC22C apparatus. The polymer samples were scanned from −100 to 150°C at a heating rate of 10°C/min under nitro-

Table II Polycondensation of Diesters with BD and Thermal Properties of Obtained Polyesters[a]

Polyester (Diester/BD)	Yield (%)	$M_n/10^4$ [b]	M_w/M_n [b]	T_g (°C)[c]	T_m (°C)[c]	$-\Delta H_m$ (J/g)[c]
DMS/BD	87.0	6.10	1.70	−30.7	115.6	74.9
DMG/BD	63.0	5.91	1.46	−53.6	43.4	45.2
DMA/BD	74.9	2.69	1.43	−56.0	61.1	80.5
DMP/BD	61.2	3.56	1.44	−55.6	46.8	53.1
DMSb/BD	85.6	5.26	1.43	−61.2	57.9	70.5
DMAz/BD	80.3	4.54	1.33	−59.4	49.4	58.6
DMSe/BD	81.0	3.78	1.56	−54.5	66.8	69.5

[a] Conditions are the same as in the Experimental section.
[b,c] Determined by GPC and DSC, respectively.

Table III Changes in Molecular Weight and Thermal Properties of Polyesters before and after Chain Extension

Polyester (Diester/BD)	Before Extension[a]				After Extension[b]			
	$M_n/10^4$ [c]	T_g (°C)[d]	T_m (°C)[d]	$-\Delta H_m$ (J/g)[d]	$M_n/10^4$ [c]	T_g (°C)[d]	T_m (°C)[d]	$-\Delta H_m$ (J/g)[d]
DMS/BD	2.60	−30.4	115.0	73.4	5.60	−32.0	117.8	78.0
DMA/BD	1.59	−56.9	58.5	79.0	3.47	−55.5	60.0	82.4
DMP/BD	2.13	−57.2	46.5	51.0	4.89	−56.9	47.5	51.8
DMSe/BD	1.85	−55.5	65.3	69.1	4.50	−56.1	65.7	70.1

[a] Reacted at 1 Torr using the molar ratio of diol to diester 1.2.
[b] Chain extended at 100°C in $CHCl_3$ *in vacuo* (ca. 50 Torr) using HDI 2 wt %/polyester.
[c,d] Determined by GPC and DSC, respectively.

gen stream. The values of T_m and ΔH_m were determined in the first heating, while that of T_g was determined in the second heating. IR spectra of polymers before and after chain extension were measured on a JASCO FT/IR-300E to confirm the bridging of polyesters by HDI.

Enzymatic Degradation of Polyesters

The enzymatic degradation was carried out as follows. The polymer films sealed in a polyethylene mesh sheet (mesh size ca. 1 × 1 mm) were placed in vial tubes containing enzyme (1 unit/mg-polyester) and buffer solution. The tubes were incubated at 37°C in a water bath shaker. The buffered enzyme solution was replaced every 40 h to maintain the enzyme activity. All the sample films (ca. 100 μm in thickness) were prepared by solvent casting method. The enzymes used were cholesterol esterase (from *Pseudomonas* sp., specific activity 20 IU/mg), lipase B (from *Pseudomonas fragi* 22–39B, specific activity 2500 IU/mg) both from Wako Pure Chemical Industries, Ltd., and lipase (from *Rhizopus delemar* (*R. delemar*), specific activity 200 IU/mg, Seikagaku Corp.). Acetate (pH 5.6, for *R. delemar* lipase) and phosphate (pH 7.2, for cholesterol esterase; pH 9.0, for lipase B) buffers were used. Distilled and deionized water was used for degradation tests. Further, water-soluble products of polyesters degraded by enzyme were isolated using a fraction collector, and their lowest molecular weight fractions were analyzed by ^{1}H-NMR spectroscopy.

In the all above degradation tests, the polymer films were washed with water at a definite time, and dried to constant weight *in vacuo*. The degradability was evaluated from the changes in weight loss, molecular weight, and thermal properties of the polymer before and after degradation. Duplicate degradation tests were carried out for each sample. The obtained values in the above measurements were within 10% from the mean.

RESULTS AND DISCUSSION

Synthesis and Characterization of Polyesters

Table I shows the results of the polycondensation of DMS with BD by various metal alkoxides. The polycondensations by $Sm(OiPr)_3$ and $Ge(OEt)_4$ yielded only the oligomers with M_n values of several hundreds to thousands. Among the catalysts used, $Ti(OiPr)_4$ is best-balanced catalyst in terms of molecular weight and yield. The electronegativity of metals and the bulkiness of alkoxy groups may affect the ease of transesterification. From the results in Table I, $Ti(OiPr)_4$ was used as catalyst in the following polycondensations.

Table II shows the results of polycondensation of various diesters with BD and the thermal properties of the resulting polyesters. The molecular weights (M_n) of polyesters except for DMA/BD polyester exceed 35,000, and are considerably higher than those (ca. 10,000–30,000) reported in our previous work.[5] Probably, the higher vacuum (0.1 Torr) and the lower molar ratio (1.1) of diol to diester compared with those in the previous work[5] brought these higher M_n values. Concerning the thermal properties, T_m values of the polyesters are rather low (43.4–66.8°C) except that (115.6°C) of DMS/BD polyester. The ΔH_m values of polyesters prepared from diesters (DMG, DMP, DMAz) with odd methylene numbers are relatively lower than those prepared from diesters (DMS, DMA, DMSb, DMSe) with even methylene numbers. This result indicates the lower crystallinity of the former polyesters.

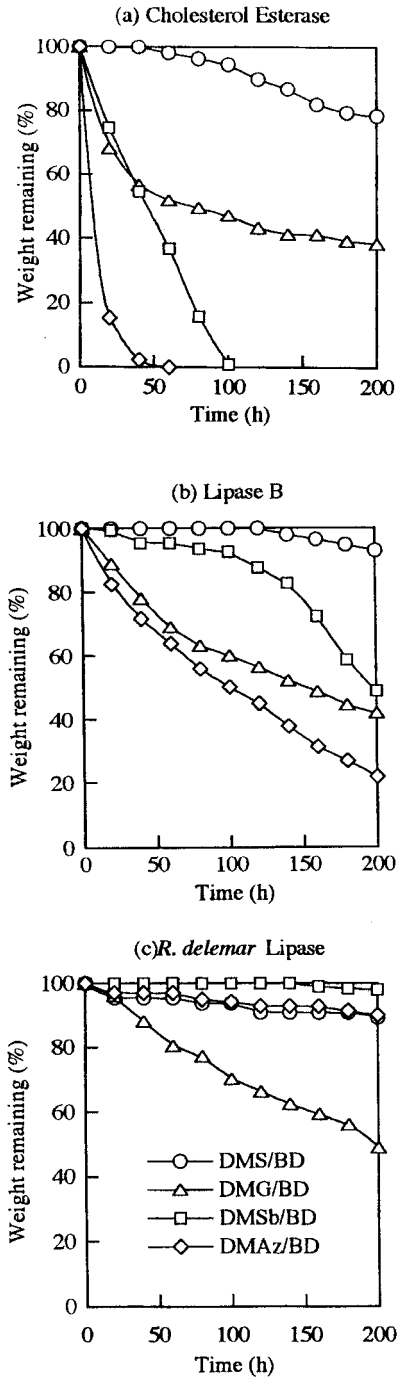


Figure 1 Enzymatic degradation of diester/BD polyesters at 37°C by three different enzymes; (a) cholesterol esterase (pH 7.2), (b) lipase B (pH 9.0), (c) *R. delemar* lipase (pH 5.6).

Chain Extension of Polyesters

It is rather difficult to obtain higher molecular weight polyesters by chain extension reaction using the polyesters with $M_n > 40000$. This is probably because that the concentrations of their terminal groups (—OH) are relatively low, and the stirring of these viscous polymer solutions become incomplete. Hence, we prepared the polyesters with lower molecular weights for chain extension by changing the final vacuum pressure 0.1 to 1.0 Torr and the molar ratio of diol to diester 1.1 to 1.2 as mentioned above. Table III shows the changes in molecular weight and thermal properties of polyesters before and after chain extension. These DMA/BD, DMP/BD, and DMSe/BD polyesters are the polymers whose molecular weights cannot be raised easily (to more than 40,000) even under the optimum conditions (see Table II). As can be seen from Table III, the M_n values of chain extended polyesters are more than two times (2.15–2.43). The DMA/BD polyester turned out to be film-formable after chain extension.

On the other hand, the thermal properties of polyesters hardly change before and after chain extension, suggesting that the chain extension reaction of these polyesters by HDI does not affect the thermal properties of chain extended polyesters. To confirm the urethane bond produced after chain extension, the IR spectra of polyesters were measured. The bending (1540 cm^{-1}) and stretching (3200–3400 cm^{-1}) vibrations of N—H in urethane bond of polyesters appeared after chain extension, accompanying a drastic decrease in O—H vibration peak (3400–3600 cm^{-1}) of polyester terminals before chain extension.

Enzymatic Degradation of Polyesters

The enzymatic degradations of polyester films were examined in buffer solutions using three different enzymes. Those enzymes were selected from the following reasons. Cholesterol esterase has a higher substrate specificity for aliphatic polyesters with relatively longer methylene chains, and hence, for more hydrophobic polyesters such as poly(ϵ-caprolactone) [poly(CL)] than those with shorter methylene chains like poly(β-propiolactone) [poly(PL)].[8] Whereas *R. delemar* lipase has the opposite specificity, i.e., the lipase degraded more poly(PL) than poly(CL).[9] The substrate specificity of lipase B is intermediate between cholesterol esterase and *R. delemar* lipase,[10] and this lipase B has cholesterol esterase activity as well. Among the polyesters obtained, the film formation of DMA/BD polyester before chain extension was unsuccessful by solvent casting method. Further, we could not weigh

Table IV Changes in Some Properties of Polyesters before and after Degradation by Cholesterol Esterase

Polyester (Diester/BD)	Degradation					
	Time (h)	Weight loss (%)	$M_n/10^4$ [a]	M_w/M_n [a]	T_m (°C)[b]	$-\Delta H_m$ (J/g)[b]
DMG/BD	0	0	5.91	1.46	43.4	45.2
	40	44.9	5.83	1.49	43.5	49.6
DMSb/BD	0	0	5.26	1.43	57.9	70.5
	40	49.8	4.98	1.59	58.1	78.2
DMAz/BD	0	0	4.54	1.33	49.4	58.6
	20	84.8	4.50	1.45	50.7	64.5

[a,b] Determined by GPC and DSC, respectively.

DMP/BD and DMSe/BD films sealed in a polyethylene mesh sheet, because these films decomposed into smaller fragments, which could pass through a mesh sheet during degradation. Thus, we did not use these three polyesters in degradation tests.

Before enzymatic degradation tests, hydrolytic degradations of the polyesters were carried out in every buffer solution containing no enzyme. Consequently, the weight losses of the polymers after 200-h degradation were only a few percent. Figure 1 shows the enzymatic degradations of aliphatic polyesters by three different enzymes. It is apparent that the degradability of polyesters by enzymes increased in the order of cholesterol esterase > lipase B > *R. delemar* lipase. As shown in Figure 1(a), cholesterol esterase degraded almost perfectly DMAz/BD and DMSb/BD polyesters after ca. 40 and 100 h, respectively. The DMG/BD polyester was degraded rapidly at first by this enzyme, but after 50 h, its degradation rate became slower. Whereas, the DMS/BD polyester was not degraded very much.

These degradation behaviors are probably correlated to chain–chain interactions, crystallinity (in other words, T_m and ΔH_m values), and molecular weights of polymers. Because the differences in molecular weights of the polyesters used in degradation tests are not very large (Table II), the degradability is probably little affected by polymer molecular weight and chain–chain interactions. The polyester with a high melting temperature like DMS/BD polyester (T_m 115.6°C) was hardly degraded by every enzyme. Tokiwa et al.[6,9,11] reported that the degradability of aliphatic polyesters ($M_n < 10000$) by lipases depended strongly on their T_m values, and the higher the T_m value was, the lesser the degradability was, especially for the polyesters whose T_m values are > 60°C. However, as shown in Figure 1, the degradations of DMG/BD, DMSb/BD, and DMAz/BD polyesters do not necessarily increase with decreasing their T_m values (see Table II). For example, cholesterol esterase degraded more DMSb/BD polyester (T_m 57.9°C) than DMG/BD polyester (T_m 43°C). As stated above, this is probably due to the substrate specificity of this enzyme. *R. delemar* lipase degraded only DMG/BD polyester moderately [Fig. 1(c)], and lipase B showed the intermediate degradability between above two enzymes [Fig. 1(b)].

Table IV shows the changes in some properties of polyesters before and after degradation by cholesterol esterase. It can be seen that the molecular weight and polydispersity of polyesters hardly changed before and after enzymatic degradation. This result indicates that the degradation of (water-insoluble or hydrophobic) polyesters occurs uniformly from their film surfaces. On the other hand, the thermal properties (especially ΔH_m values) of polyesters increased after degradation, suggesting that the enzymatic degradation would occur preferentially in amorphous parts of polyesters[12–14] and/or the partial crystallization of amorphous region during degradation.[15]

Figure 2 shows the ^{1}H-NMR spectrum of DMG/BD polyester before degradation and that of the lowest molecular weight fraction of water-soluble products degraded by cholesterol esterase at 40 h. It is clear that the new peak (a′), corresponding to α-methylene proton peak of alcohol, appears around 3.7 ppm after degradation [Fig. 2(b)]. Considering from the chemical shifts and integral values of those peaks, the lowest molecular weight fraction of water-soluble degraded products for DMG/BD polyester is the mixture of

BD and the condensation product of DMG with BD. Tsuji et al.[16] also reported that the water-soluble degraded product of poly(hexamethylene adipate) was the condensation product of adipic acid with hexamethylene glycol.

Among chain-extended samples in Table III, DMS/BD polyester was the only polymer film whose weight loss by enzymatic degradation could be measured before and after chain extension. Figure 3 shows enzymatic degradation of DMS/BD polyester films by cholesterol esterase before and after chain extension. The degradability of the chain extended film by the esterase is slightly smaller than that of the unextended one. With an increase of molecular weight of the polymer, intra- and intermolecular entanglements increase, leading to the difficulty in enzymatic degradation of chain extended polyesters. Further, the urethane bond in chain extended polyesters, which may not be cleaved by the esterase, will retard the degradation rate. In fact, it is reported that the enzymatic degradability of polyurethanes was lower compared with that of the original polyesters.[17] However, we confirmed that in more prolonged degradations up to 900 h, chain extended polyesters were degraded slowly but steadily.[18] As can be seen from Figure 3, the chain extended DMA/BD polyester film, having much lower T_m value than the DMS/BD polyester, was completely degraded after 60 h.

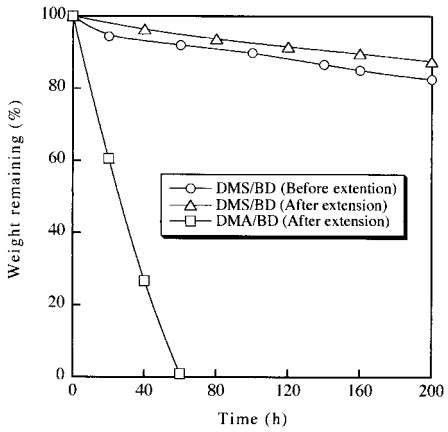


Figure 3 Enzymatic degradation of DMS/BD polyesters by cholesterol esterase before and after chain extension (pH 7.2, 37°C).

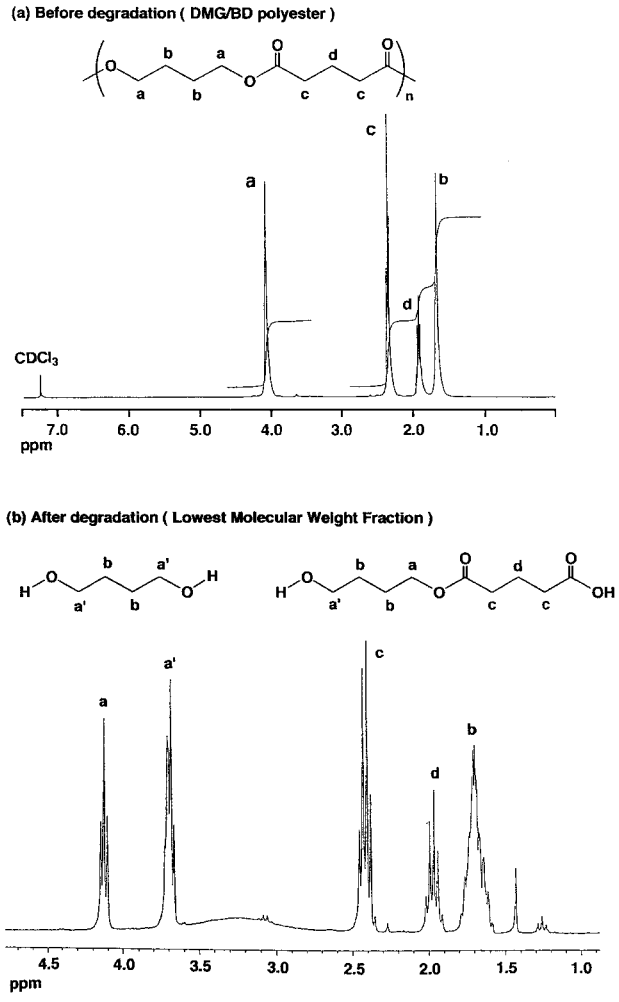


Figure 2 ^{1}H-NMR spectra of DMG/BD polyester (a) and the lowest molecular weight fraction (b) of water-soluble products degraded by cholesterol esterase after 40 h.

This work was supported by a Grant-in-Aid for Scientific Research on Priority Area, "Sustainable Biodegradable Plastics," No. 11217211 (1999) from the Ministry of Education, Science, Sports and Culture (Japan).

REFERENCES

1. Aida, T.; Sanuki, K.; Inoue, S. Macromolecules, 1985, 18, 1049.
2. Nakayama, A.; Kawasaki, N.; Hayashi, K.; Yamamoto, N.; Maeda, Y. Polym Prep Jpn 1993, 42, 3739.
3. Kobayashi, S.; Uyama, H. Makromol Chem Rapid Commun 1993, 14, 841.
4. Migita, N. Graduation Thesis, Hiroshima University, 1995.
5. Shirahama, H.; Aludin, M. S.; Kawaguchi, Y.; Migita, N.; Yasuda, H. J Jpn Oil Chem Soc 1997, 46, 1391.
6. Tokiwa, Y.; Ando, T.; Suzuki, T.; Takeda, K. ACS Sym Series 1990, 433, 136.

7. Rehman, I.; Andrews, E. H.; Smith, R. J Mater Sci Mater Med 1996, 7, 17.
8. Shirahama, H.; Mizuma, K.; Kawaguchi, Y.; Shiomi, M.; Yasuda, H. Kobunshi Ronbunshu (Jpn J Polym Sci Technol 1993, 50, 821.
9. Tokiwa, Y.; Suzuki, T. Agric Biol Chem 1978, 42, 1071.
10. Kawaguchi, Y. Master's Thesis, Hiroshima University, 1995.
11. Tokiwa, Y.; Suzuki, T.; Takeda, K. Agric Biol Chem 1986, 50, 1323.
12. Kumagai, Y.; Doi, Y. Makromol Chem 1991, 193, 53.
13. Li, S. M.; Gerreau, H.; Vert, M. J Mater Sci Mater Med 1990, 1, 198.
14. Shirahama, H.; Umemoto, K.; Yasuda, H. J Biomater Sci Polym Ed 1999, 10, 621.
15. Tsuji, H.; Ikada, Y. J Polym Sci Part A Polym Chem 1998, 36, 59.
16. Tsuji, M.; Ichikawa, Y. Hakko Kogaku 1978, 56, 799.
17. Tokiwa, Y.; Suzuki, T.; Takeda, K. Agric Biol Chem 1988, 52, 1937.
18. Shirahama, H.; Hori, H.; Yasuda, H. J Jpn Oil Chem Soc 1999, 48, 911.