Enzymatic Synthesis of Polythioester by the Ring-Opening Polymerization of Cyclic Thioester

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A high molecular weight aliphatic polythioester was prepared by lipase-catalyzed polymerization of hexane-1,6-dithiol and dimethyl sebacate using the technique of ring-opening polymerization of a cyclic thioester. The cyclic thioester monomer was first prepared using lipase from *Candida antarctica* in dilute solution. The monomer was then polymerized by the same lipase in bulk to produce a polythioester with an M_w of about 120 000 g/mol, which was significantly higher than that of a polythioester obtained by direct polycondensation of the dithiol and diacid. The polymerization rate and thermal properties of the product were measured and compared with those of the corresponding polyester prepared by ring-opening polymerization of a cyclic ester.

Introduction

Polymers containing backbone sulfur atoms are of interest as many of their properties are superior to those of the corresponding polyesters, e.g., higher melting temperature, greater heat stability, and lower solubility in various organic solvents. For this reason, a variety of sulfur-containing polymers have been considered as engineering plastics. Poly(phenylene sulfide) is a good example; its melting temperature of over 300 °C is higher than that of poly(phenylene oxide) (262 °C).

In addition to polythioethers, polythioesters have been a subject of attention since the recent discovery of microbial production of poly(3-mercaptoalkanoate)s. It was reported by Lütke-Eversloh and co-workers that when engineered *Escherichia coli* was cultured with mercaptoalkanoic acids, such as 3-mercaptopropionic acid, copolymers containing thioester linkages in their backbones were produced.[1,2] They also reported the synthesis of novel homopolythioesters using a recombinant strain of *E. coli* and characterized their properties.[3,4]

Although a chemical synthesis of polythioesters was first reported in 1951, few reports have been published on the synthesis of aliphatic polythioesters, partly due to their complicated synthetic procedure.[5−8] In the conventional process, the requirement for toxic reagents is also a negative factor. Enzyme-catalyzed polymerization, in which the use of acid chlorides and toxic organic solvents is avoided, is potentially an environmentally benign method for industrial production of polythioesters.[9] With the use of an enzyme catalyst, the thioesterification reaction may be realized under relatively mild conditions.

There have been many reports on the synthesis of polyesters, polycarbonates, and polyamides using enzymes.[10,11] The formation of thioester linkages by condensation of alkanethiols and carboxylic acids or carboxylic acid esters using an enzyme has also been reported.[12−14] The introduction of a thioester linkage into a polymer chain via an enzymatic reaction was first performed by Iwata et al.[15] We previously reported lipase-catalyzed polycondensation of 11-mercaptoundecanoic acid to form a polythioester with an M_w of 34 000 g/mol.[16] With the aim of preparing a variety of polythioesters using commercially available monomers, we also synthesized polythioesters by direct polycondensation of a diacid diester and a dithiol. The resulting polythioesters showed higher melting temperatures than the corresponding polyesters.[17] However, due to its low molecular weight, the obtained polythioester was powder-like and could not form a film. A polythioester with an M_w greater than 100 000 g/mol is required in order to obtain satisfactory mechanical properties, such as tensile strength, elongation-to-break, and film formation ability.

Fehling et al. have studied the use of chemoenzymatic reactions (lipase-catalyzed thioesterification followed by thiyl-radical-induced addition to the C═C double bond) in order to increase the molecular weight of the polythioester. The resulting polythioester was a mixture of several chemical structures with molecular weights of about 2000 g/mol.[18] We previously reported a strategy for the production of a higher molecular weight diol−diacid-type polyester via an enzymatic reaction. That is, lipase-catalyzed polymerization involving formation of cyclic oligomers and subsequent ring-opening polymerization of the cyclic oligomer was carried out.[19] The ring-opening polymerization reaction suppressed the reverse reaction resulting from the presence of polycondensation products such as water, thus resulting in a higher molecular weight. In this study, this strategy was applied to lipase-catalyzed polymerization of a dithiol and a diacid diester in order to produce a high molecular weight polythioester. The enzymatic reactivity of the cyclic thioester was also studied and compared to that of a cyclic ester in order to clarify the mechanism of ring-opening polymerization. The mechanistic aspects of lipase-catalyzed ring-opening polymerization have been the subject of much investigation and are indispensable for increasing our understanding of enzymatic polymerization.[20,21] The reactivity of the mercapto group toward the enzyme-activated monomer was also investigated.

Experimental Section

Materials and Measurements. Hexane-1,6-dithiol and 1-propanol (anhydrous) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Hexane-1,6-diol, *n*-nonane, dimethyl sebacate, chloroform (stabilized with amylene), and toluene were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Immobilized lipase from *Candida antarctica* [lipase CA: Novozym 435, a lipase (B lipase) from *C. antarctica* produced by submerged fermentation of a genetically

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modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin, having 10 000 PLU·g^{-1} (propyl laurate units: lipase activity based on ester synthesis)] was supplied by Novozymes Japan Ltd. (Chiba, Japan). The enzyme was dried under vacuum (3 mmHg) over P_2O_5 at 25 °C for 2 h before use. Thermally deactivated lipase CA was prepared by heating the enzyme in steam using an autoclave at 120 °C for 15 min and then freeze-drying in vacuum.

Molecular sieves 4A were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). The molecular sieves 4A were dried at 150 °C for 2 h before use.

The weight-average (M_w) and number-average (M_n) molecular weights as well as molecular weight distribution (M_w/M_n) of the polymer were determined by a size exclusion chromatography (SEC) using SEC columns (Shodex K-G + K-804, Showa Denko Co., Ltd., Tokyo, Japan) with a refractive index detector. Chloroform was used as the eluent at 1.0 mL·min^{-1}. The SEC system was calibrated with polystyrene standards of a narrow molecular weight distribution.

The absolute molecular weight was measured by a multiangle laser light scattering (MALS) detector (DAWN EOS, Wyatt Technology Corp., Santa Barbara, CA) and a differential refractrometer for the determination of the d*n*/d*c* value (DRM-3000, Otsuka Electronics Co., Ltd., Osaka, Japan). Chloroform was used as the solvent.

The conversion of the cyclic monomer was determined on the basis of the peak area of the SEC using an SEC column (Shodex K-801, Showa Denko Co., Ltd., Tokyo, Japan). Chloroform was used as the eluent at 1.0 mL·min^{-1}. The SEC system was calibrated with the cyclic monomer. Through this SEC column, monomer peak was completely separated from polymer peaks. First, we made calibration curves (cyclic monomer concentration vs peak area). Both of these curves showed a linear structure with an $R^2 > 0.99$. The SEC measurement of the reacted sample has always been taken in a constant concentration (3 mg/mL). The conversion was determined using the calibration curve. The retention time for the cyclic monomer is as follows: cyclic(hexanedithiol-sebacate), 7.1 min; cyclic(hexanediol-sebacate), 6.9 min

The atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was measured with Finnigan Mat Inc. LCQ-mass spectrometer system (Finnigan Corp., San Jose, CA).

The ^{1}H and ^{13}C NMR spectra were recorded on a Lambda 300 Fourier transform spectrometer (JEOL Ltd., Tokyo, Japan) operating at 300 MHz and 75 MHz, respectively.

The water contents were measured using a Karl Fischer coulometer (831 KF coulometer, Metrohm, Herisau, Switzerland).

The crystallization temperature (T_{cc}) and melting temperature (T_m) of the polymer were determined by a differential scanning calorimetry (DSC-60, Shimadzu, Kyoto, Japan). The measurements were made with a 3−5 mg sample on a DSC plate. The polymer samples were heated at the rate of 10 °C·min^{-1} from 30 to 150 °C (first scan), rapidly cooled to −80 °C at the rate of 50 °C·min^{-1}, and then scanned at the same heating rate of 10 °C and over the temperature range of −80 to 150 °C (second scan). The T_{cc} was collected from the cooling stage, and the T_m and fusion enthalpy per unit (ΔH_u) were collected from the second scan. The fusion entropy per unit (ΔS_u) was calculated from the T_m and ΔH_u.

The decomposition temperature (T_d) and the decomposition temperature at 5 wt % weight loss ($T_{d(5\%)}$) were determined by a thermogravimetric analysis (DTG-60, Shimadzu, Kyoto, Japan). The measurements were made with a 2−4 mg sample. The polymer samples were heated from room temperature to 600 °C at the rate of 10 °C·min^{-1}.

General Enzymatic Cyclization Procedure. The general procedure for the cyclization was carried out in a round-bottom flask with molecular sieves 4A placed at the top of the flask in the vapor phase to absorb the produced methanol. A typical procedure for the reaction was as follows. A mixture of hexane-1,6-dithiol (0.40 mmol), dimethyl sebacate (0.40 mmol), immobilized lipase CA (184.4 mg), and *n*-nonane (20 mL) was stirred using a magnetic stirring bar under a nitrogen atmosphere in a thermostated oil bath at 120 °C for 24 h. The reaction mixture was dissolved in chloroform, and insoluble enzyme was removed by filtration through a Celite pad. The solvent was then evaporated under reduced pressure to obtain the crude mixture. The crude products were further purified by silica gel column chromatography using ethyl acetate/hexane (1:12, v/v; $R_f = 0.28$) as the eluent to obtain the cyclic(hexanedithiol-sebacate) monomer in a 16.4% yield. The molecular structure of the product was analyzed by ^{1}H NMR spectroscopy, SEC, and elemental analysis. The spectral data of cyclic(hexanedithiol-sebacate) monomer are as follows. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.20 ∼ 1.33 (m, 8H, $-CH_2CH_2CH_2CH_2COS-$), 1.34 ∼ 1.41 (m, 4H, $-SCH_2CH_2CH_2-$), 1.51 ∼ 1.62 (m, 4H, $-CH_2CH_2CH_2CH_2COS-$), 1.62 ∼ 1.74 (m, 4H, $-SCH_2CH_2CH_2-$), 2.54 (t, 4H, $-CH_2CH_2CH_2CH_2COS-$, J = 6.2 Hz), 2.85 (t, 4H, $SCH_2CH_2CH_2-$, J = 6.6 Hz). $(C_{16}H_{28}O_2S_2)_n$ (316.52)$_n$ Calcd: C, 60.71; H, 8.92; S, 20.26. Found: C, 60.83; H, 9.01; S, 20.10.

Using a similar procedure, the cyclic(hexanediol-sebacate) was prepared by the reaction of hexane-1,6-diol (0.40 mmol) and dimethyl sebacate (0.40 mmol) with lipase CA (184.4 mg) in *n*-nonane (20 mL). Purification was carried out by silica gel column chromatography using ethyl acetate/hexane (1:6, v/v; $R_f = 0.38$) as the eluent to obtain the cyclic(hexanediol-sebacate) monomer in a 77.5% yield. The spectral data of cyclic(hexanediol-sebacate) monomer are as follows. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.24 ∼ 1.34 (m, 8H, $-CH_2CH_2CH_2CH_2COO-$), 1.44 (tt, 4H, $-OCH_2CH_2CH_2-$, J = 3.6, 3.6 Hz), 1.58 ∼ 1.72 (m, 8H, $-OCH_2CH_2CH_2-$, $-CH_2CH_2CH_2CH_2COO-$), 2.32 (t, 4H, $-CH_2CH_2CH_2CH_2COO-$, J = 6.5 Hz), 4.13 (t, 4H, $-OCH_2CH_2CH_2-$, J = 5.7 Hz). $(C_{16}H_{28}O_4)_n$ (284.39)$_n$ Calcd: C, 67.57; H, 9.92. Found: C, 67.73; H, 9.84.

General Enzymatic Polymerization Procedure of Cyclic Monomer. The general procedure for the ring-opening polymerization of a cyclic thioester monomer was carried out in a round-bottom flask with molecular sieves 4A placed at the top of the flask in the vapor phase. A typical procedure for the reaction was as follows. A mixture of the cyclic monomer (0.063 mmol) and immobilized lipase CA (14 mg) was placed under a nitrogen atmosphere in a thermostated oil bath at 120 °C for 48 h. The reaction mixture was dissolved in 15 mL of hot chloroform at 50 °C, and the insoluble enzyme was removed by filtration. The solvent was then evaporated to obtain the crude polymer. The crude polymer was purified by reprecipitation using chloroform (good solvent)−methanol (poor solvent) to obtain poly(hexanedithiol-sebacate) with M_w = 115 000 g/mol and M_w/M_n = 2.3 in a 90.1% yield. The molecular weight and the molecular weight distribution of the polymer were determined using SEC. The molecular structure was analyzed by ^{1}H NMR spectroscopy. The spectral data of poly(hexanedithiol-sebacate) are as follows. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.22 ∼ 1.33 (m, 8H, $-CH_2CH_2CH_2CH_2COS-$), 1.34 ∼ 1.44 (m, 4H, $-SCH_2CH_2CH_2-$), 1.51 ∼ 1.62 (m, 4H, $-CH_2CH_2CH_2CH_2COS-$), 1.62 ∼ 1.74 (m, 4H, $-SCH_2CH_2CH_2-$), 2.53 (t, 4H, $-CH_2CH_2CH_2CH_2COS-$, J = 7.5 Hz), 2.85 (t, 4H, $SCH_2CH_2CH_2-$, J = 7.3 Hz).

Using a similar procedure, poly(hexanediol-sebacate) as the polyester reference was prepared by the ring-opening polymerization of cyclic(hexanediol-sebacate) (0.070 mmol) with immobilized lipase CA (14 mg) at 120 °C for 48 h. Poly(hexanediol-sebacate) with M_w = 219 000 g/mol and M_w/M_n = 2.1 was obtained in a 94.3% yield. The spectral data of poly(hexanediol-sebacate) are as follows. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 1.24 ∼ 1.34 (m, 8H, $-CH_2CH_2CH_2CH_2COO-$), 1.35 ∼ 1.48 (m, 4H, $-OCH_2CH_2CH_2-$), 1.51 ∼ 1.72 (m, 4H, $-OCH_2CH_2CH_2-$), 2.32 (t, 4H, $-CH_2CH_2CH_2CH_2COO-$, J = 6.5 Hz), 4.13 (t, 4H, $OCH_2CH_2CH_2-$, J = 5.7 Hz).

General Reaction of Cyclic Monomer with 1-Propanol as a Nucleophile. The general procedure for the determination of the cyclic monomer conversion with 1-propanol as a nucleophile was carried out in a dried round-bottom flask. A typical procedure for the reaction was as follows. A mixture of the cyclic monomer (0.063 mmol), anhydrous toluene (63.2 μL), and 1-propanol (0.063 mmol) was placed under a

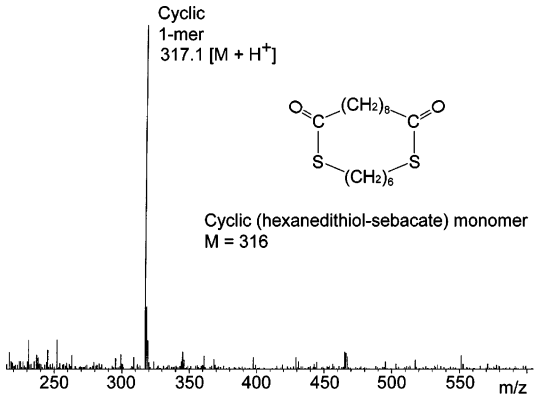


Figure 1. APCI-MS spectrum of the cyclization product after purification by silica gel column chromatography. Reaction conditions: cyclization of hexane-1,6-dithiol and dimethyl sebacate was carried out using 200 wt % lipase CA in *n*-nonane at 120 °C for 24 h.

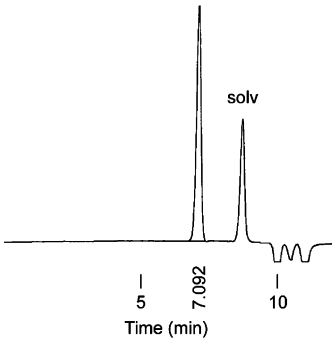


Figure 2. SEC profile of cyclic(hexanedithiol-sebacate) monomer.

nitrogen atmosphere in a thermostated oil bath at 50 °C. After the reactor reached 50 °C, the lipase CA (2 mg) was quickly added to the round-bottom flask. The reaction mixture was then stirred using a magnetic stirring bar at 50 °C for 1 to ~5 min. The reaction was stopped by dissolving the mixture with chloroform and quickly removing the insoluble enzyme by filtration. In a similar procedure, the reaction was carried out without 1-propanol as the reference. These reactions were also carried out using the cyclic ester monomer instead of the cyclic thioester monomer.

Results and Discussion

Lipase-Catalyzed Synthesis of Cyclic Thioester Monomer. First, hexane-1,6-dithiol and dimethyl sebacate were reacted with lipase CA in a dilute *n*-nonane solution. It was found that in dilute solution, hexane-1,6-dithiol and dimethyl sebacate were transformed into the cyclic thioester monomer. The ^{1}H NMR spectrum of the product after purification by silica gel column chromatography using ethyl acetate/hexane showed that the peak ascribed to the linear structure ($\delta = 3.67$, s, 3H) was not present. Before purification, some side reaction products with various chemical structures, such as a linear monomer and dimer, were detected by ^{1}H NMR. In order to confirm the ring size of the resulting cyclic monomer, the APCI-MS spectrum of the purified product was measured (Figure 1). It was confirmed that a single, sharp peak was observed at $m/z = 317.1$, which is equivalent to the molecular mass of the cyclic(hexanedithiol-sebacate) monomer. Figure 2 shows the SEC profile of the purified cyclic monomer. A single, sharp peak attributed to the cyclic monomer was observed at a retention time of 7.1 min.

In a similar procedure, a cyclic(hexanediol-sebacate) monomer was prepared by reaction of hexane-1,6-diol and dimethyl sebacate in *n*-nonane using lipase CA. After purification by silica gel column chromatography, the molecular structure was confirmed using ^{1}H NMR, APCI-MS, and SEC. No peaks due to the linear structure were detected in the ^{1}H NMR spectrum, and a single, sharp peak was observed for the molecular mass at *m*/*z* 285.0, equivalent to that of the cyclic(hexanediol-sebacate) monomer. In addition, SEC analysis showed a single, sharp peak for the cyclic ester monomer at a retention time of 6.9 min.

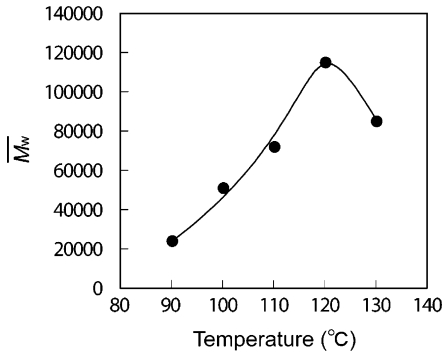


Figure 3. Effect of temperature on M_w of poly(hexanedithiol-sebacate). Reaction conditions: cyclic(hexanedithiol-sebacate) (0.063 mmol) was polymerized by lipase CA (70 wt %) in bulk with molecular sieves 4A at the top of the vial for 48 h.

Ring-Opening Polymerization of Cyclic Monomers. We previously reported that lipase CA showed the best result among various lipases tested for enzyme-catalyzed polycondensation of diacid diesters and dithiols.[17] Therefore, in this study, lipase CA was used for ring-opening polymerization of the cyclic thioester.

It was confirmed that the molecular weight of the resulting polymer was influenced by the polymerization conditions, such as reaction temperature, enzyme concentration, and reaction time. Figure 3 shows molecular weight as a function of polymerization temperature for 48 h polymerization of the cyclic(hexanedithiol-sebacate) monomer using 70 wt % lipase CA (based on the wt % of the immobilized lipase) in bulk. It was found that the polythioester with the highest molecular weight was produced at 120 °C. At temperatures higher than 120 °C, the molecular weight decreased due to deactivation of the enzyme. The reaction temperature of 120 °C is higher than that used in conventional aqueous solution reactions, but some reports have indicated that under relatively anhydrous conditions, enzymes such as lipases, proteases, and esterases are catalytically active at temperatures around 90−130 °C.[22−25] The same tendency was observed in the synthesis of polythioesters by direct polycondensation, as we reported previously. It was also confirmed that no reaction occurred under the same conditions in the absence of lipase CA or when using thermally deactivated lipase CA, which indicates that ring-opening polymerization of cyclic(hexanedithiol-sebacate) is caused by lipase CA. At lower reaction temperatures, nucleophilic attack of the acyl-enzyme intermediate by the mercapto group may be disturbed, which leads to a product with lower molecular weight. Enzymatic reactions of mercapto-containing compounds at 60−80 °C have been reported to form mercapto end-functionalized polymers.[26] For example, Hedfors et al. reported the mercapto functionalization of poly(ϵ-caprolactone) by enzymatic ring-opening polymerization of ϵ-caprolactone at 60 °C using 2-mercaptoethanol as an initiator.[27] These reports indicated that nucleophilic attack on the acyl-enzyme intermediate by a hydroxyl group is more effective than by a mercapto group.

The M_w of poly(hexanedithiol-sebacate) produced by enzymatic ring-opening polymerization of the cyclic(hexanedithiol-

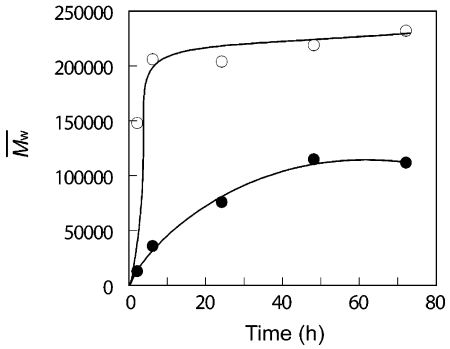


Figure 4. Time courses for polymerization of cyclic(hexanedithiol-sebacate) and cyclic(hexanediol-sebacate): ●, cyclic(hexanedithiol-sebacate); ○, cyclic(hexanediol-sebacate). Reaction conditions: cyclic monomer (0.063 mmol) was polymerized by lipase CA (70 wt %) in bulk at 120 °C with molecular sieves 4A at the top of the vial.

sebacate) monomer was significantly higher than that of the polymer produced by enzymatic direct polycondensation of a dithiol and a diacid diester. Direct polycondensation of hexane-1,6-dithiol and dimethyl sebacate yielded a poly(hexanedithiol-sebacate) with an M_w of 10 000 g/mol after 48 h; this is about 1/10th the M_w of the polymer produced by the ring-opening polymerization method, in which the M_w was 115 000 g/mol. It is thought that since ring-opening polymerization occurs without the production of methanol as a condensation product, the reverse reaction is suppressed, which results in the formation of a product with a higher molecular weight. In the polycondensation of hexane-1,6-dithiol and dimethyl sebacate by lipase, the terminal mercapto group is successively reacted with the enzyme-activated monomer (EAM). However, compared to the more bulky mercapto group, the hydroxyl group of methanol reacts more quickly with EAM, terminating polymerization. Therefore, a polythioester with a considerably greater molecular weight is produced by ring-opening polymerization due to the absence of methanol. It has previously been reported that compared to polycondensation, ring-opening polymerization of lactones produced a product with a higher molecular weight as well as a higher monomer conversion. Sugihara et al. reported that ring-opening polymerization of a cyclic(butylene-succinate) oligomer yielded a poly(butylene succinate) (PBS) with an M_w of 130 000 g/mol, which was about 3 times larger than that of the polymer produced by direct polycondensation of the two substrates.[28] Poly(hexanedithiol-sebacate) (M_w = 10 000 g/mol) synthesized by the direct polycondensation method was obtained in the form of a white powder due to its low molecular weight, whereas poly(hexanedithiol-sebacate) (M_w = 115 000 g/mol) synthesized by ring-opening polymerization formed a film.

The absolute molecular weights of poly(hexanedithiol-sebacate) and poly(hexanediol-sebacate) were measured using MALS and compared to those measured by SEC calibrated with polystyrene standards. Similar molecular weights were obtained using both methods; e.g., for poly(hexanedithiol-sebacate), M_w was 85 000 g/mol by MALS and 89 000 g/mol by SEC. Therefore, in this report, further measurements were obtained using the more convenient SEC method.

In order to compare the enzymatic polymerizability of the two cyclic monomers, cyclic(hexanedithiol-sebacate) and cyclic(hexanediol-sebacate) were each reacted with lipase CA under the same conditions. Figure 4 shows the M_w time courses for the products obtained by enzymatic ring-opening polymerization of cyclic monomers using 70 wt % lipase CA at 120 °C in bulk. It was found that cyclic(hexanediol-sebacate) was polymerized significantly faster than cyclic(hexanedithiol-sebacate). Cyclic-

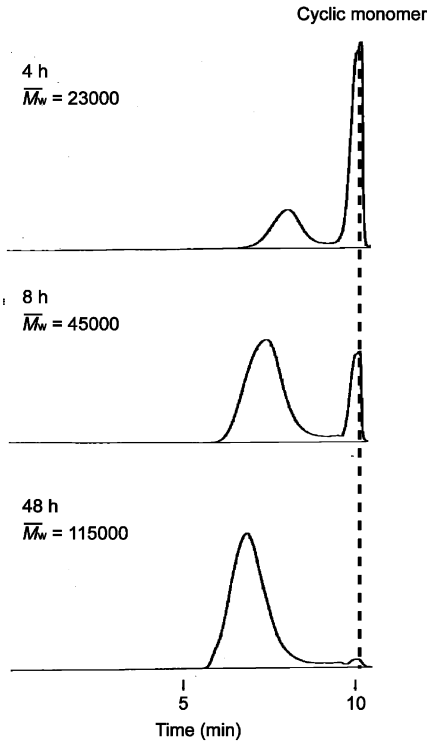


Figure 5. Time course for SEC profile changes during reaction to form poly(hexanedithiol-sebacate). Reaction conditions: cyclic(hexanedithiol-sebacate) monomer was polymerized using lipase CA (70 wt %) in bulk at 120 °C with molecular sieves 4A at the top of the vial.

(hexanediol-sebacate) produced a polymer with an M_w greater than 200 000 g/mol after a 6 h reaction, while cyclic(hexanedithiol-sebacate) required a 48 h reaction to attain an M_w of 100 000 g/mol. Figure 5 shows changes in the GPC profile during ring-opening polymerization of cyclic(hexanedithiol-sebacate). As the monomer peak gradually decreases with time, the molecular weight of the polymer increases; however, after the monomer peak is reduced almost completely, the molecular weight increases only slightly.

Figure 6a shows the time course for monomer conversion by enzymatic ring-opening polymerization of a cyclic thioester and a cyclic ester using 70 wt % lipase CA at 120 °C in bulk. Cyclic(hexanediol-sebacate) reacted quickly, with a monomer conversion of 67% after only 40 s; after 2 h, the monomer conversion exceeded 95%. In contrast, the polymerization rate of cyclic(hexanedithiol-sebacate) was quite slow, requiring 48 h to reach 90% conversion. The M_w of the polythioester gradually increased as the monomer conversion increased. The propagation rates of the cyclic monomers (R_P), derived from the slope of the time versus conversion plot assuming first-order kinetics, were 6030 h^{-1} for cyclic(hexanediol-sebacate) and 0.0137 h^{-1} for cyclic(hexanedithiol-sebacate); in other words, the polymerization rate of the cyclic thioester is about 4.4×10^5 times slower than that of the corresponding cyclic ester.

Interestingly, cyclic(hexanedithiol-sebacate) polymerized much more quickly during the initial stage than during the propagation stage. It can be seen in Figure 6a that the monomer conversion almost reached 30% within the first 2 h, after which time the polymerization rate decreased significantly. Figure 6b shows the time course for monomer conversion over the first 1 min, using an expanded scale. It was found that the cyclic monomer quickly underwent ring-opening within the first 20 s (up to 27% conversion), after which the reaction rate decreased significantly

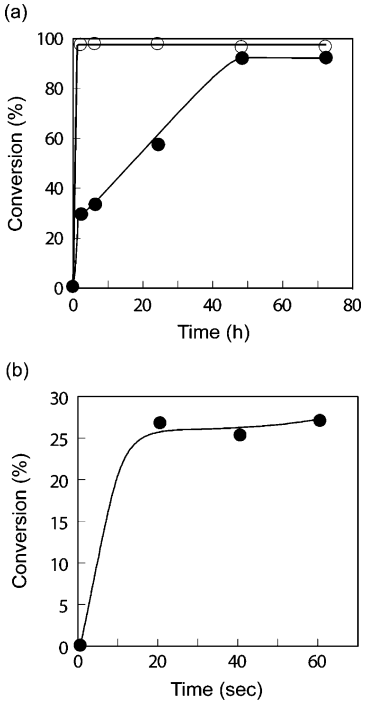


Figure 6. (a) Monomer conversion of cyclic(hexanedithiol-sebacate) and cyclic(hexanediol-sebacate); (b) monomer conversion of cyclic(hexanedithiol-sebacate) during the initial stage of the reaction: ●, cyclic(hexanedithiol-sebacate); ○, cyclic(hexanediol-sebacate). Reaction conditions: cyclic monomer (0.063 mmol) was polymerized by lipase CA (70 wt %) in bulk at 120 °C with molecular sieves 4A at the top of the vial.

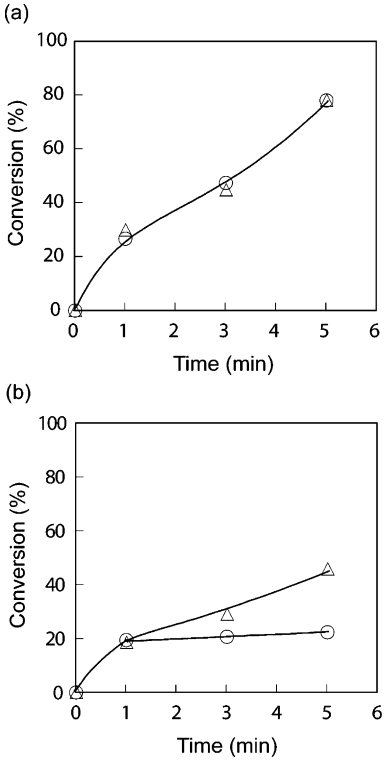


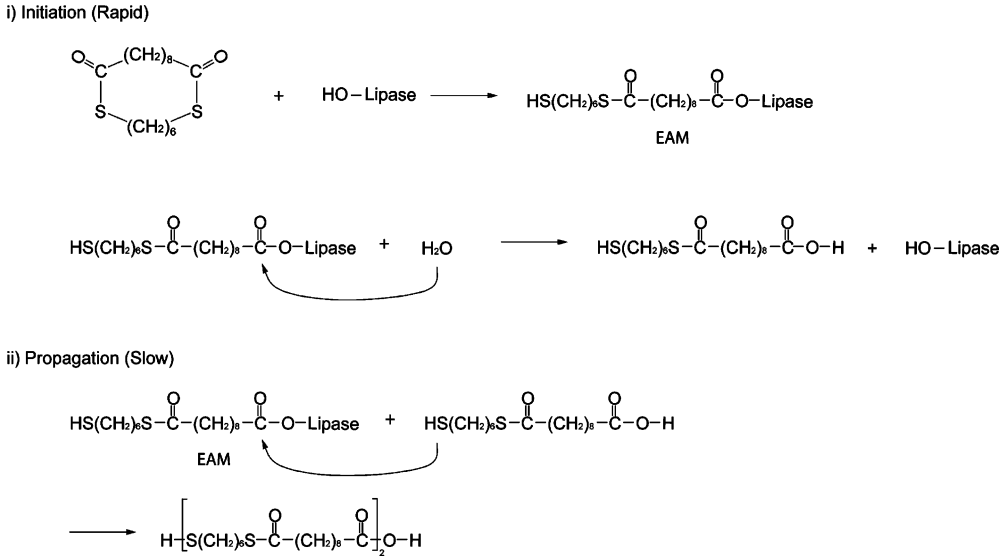
Figure 7. Conversion time course for enzymatic ring-opening reaction of (a) cyclic(hexanediol-sebacate) and (b) cyclic(hexanedithiol-sebacate) with and without 1-propanol as a nucleophile: ○, without 1-propanol; △, with 1-propanol. Reaction conditions: cyclic(hexanediol-sebacate) (0.063 mmol) or cyclic(hexanedithiol-sebacate) (0.063 mmol) was reacted with lipase CA at 50 °C in toluene (63.2 μL) with and without 1-propanol (0.063 mmol) as a nucleophile.

but remained linear up to 93% conversion. That is, the rate changed from the initial fast reaction to a slow reaction.

In order to further investigate this phenomenon, a ring-opening reaction of the cyclic monomer was carried out using 1-propanol as a nucleophile in toluene solution. Figure 7 shows the time course for monomer conversion during enzymatic ring-opening of the cyclic monomer using 20 wt % lipase CA at 50 °C in toluene (1 M), with or without 1-propanol as a nucleophile. It was found that the presence of 1-propanol did not influence the reaction rate of the cyclic ester, which indicates that the rate-determining step for polymerization of the cyclic ester is the formation of the EAM. Some previously reported mechanistic studies of lipase-catalyzed ring-opening polymerization of lactones showed similar results;[29,30] for example, Uyama et al. reported that the R_p for lipase-catalyzed polymerization of 12-dodecanolide proceeds independently of the nucleophile concentration;[31] it was also reported that the rate-determining step for ring-opening polymerization is the formation of EAM. The ring-opening reaction of the cyclic thioester, in contrast, was highly influenced by the presence of the hydroxyl-type nucleophile 1-propanol. Without 1-propanol, rapid monomer conversion stopped within 1 min, with approximately 20% conversion, while in the presence of 1-propanol, monomer conversion continued to increase. This indicates that the rate-determining step in the polymerization of cyclic(hexanedithiol-sebacate) is deacylation of the EAM via nucleophilic attack by the mercapto group. In the absence of an added nucleophile, the reaction of the monomer occurred quickly during the initial stages of the reaction due to the presence of small amounts of water in both the lipase and the substrate, which acted as a nucleophile. After this water was consumed, however, nucleophilic attack by the terminal mercapto group took place; this is significantly slower than attack by the hydroxyl group of an alcohol (Scheme 1). This may be a characteristic feature of enzymatic reactions, because the nucleophilicity of mercapto groups is generally higher than that of the hydroxyl group of an alcohol. This is probably due to efficient incorporation of the relatively bulky mercapto group into the active site of the lipase, which might be hindered when the smaller hydroxyl group is present. This is thought to be the reason for the decrease in the polymerization rate after the initial fast reaction with water. Peeters et al. demonstrated that in contrast to unsubstituted lactones, the rate-determining step for ring-opening polymerization of a 4-substituted ϵ-caprolactone is deacylation of the EAM by the propagating alcohol chain end.[32]

As shown in Scheme 1, the proposed mechanisms for the lipase-catalyzed polymerization of cyclic thioester proceeded via EAM similar to the mechanisms for lactones presented by Uyama et al.[33] The initiation is the nucleophilic attack by water on the acyl carbon of the EAM, yielding ω-mercaptocarboxylic acid, which is regarded as the basic propagation species. The EAM is successively and nucleophilically attacked by the terminal mercapto group of the growing polymer species as the propagation step. As discussed in this paper, the initiation occurred quickly; however, the propagation proceeded slowly. In order to further analyze the relative reactivity of the lipase with the cyclic thioester and the corresponding ester, a mixture of cyclic thioester and cyclic ester was polymerized by lipase CA at 120 °C in bulk (Scheme 2). Initial molar ratio of cyclic thioester and cyclic ester varied from 1:9 to 9:1. The polymerization was stopped at an early stage within 0.5−6 h depending on the molar ratio of the two monomers. After reprecipitation, the composition of the copolymer was determined by the peak area ratio of methylene protons adjacent to thioester ($\delta = 2.85$) and methylene protons adjacent to ester ($\delta = 4.13$) in ^{1}H NMR. It was found that the thioester content in the copolymer was

Scheme 1. Proposed Mechanism for Enzymatic Ring-Opening Polymerization of Cyclic(hexanedithiol-sebacate)



Scheme 2. Ring-Opening Copolymerization of Cyclic Thioester and Cyclic Ester Using Lipase CA

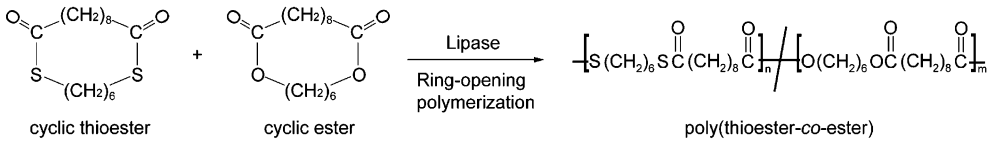


Table 1. Thermal Properties of Poly(thioester) and Poly(ester)

polymer	M_w	M_w/M_n	T_g (°C)	T_{cc} (°C)	T_m (°C)	$T_{d(5\%)}$ (°C)	ΔH_u (kJ/mol)	ΔS_u (J/mol/K)
poly(hexanedithiol-sebacate)	100 000	2.1		85.6	108.8	338.5	14.5	38.0
poly(hexanediol-sebacate)	100 000	2.0		40.0	74.8	338.5	17.3	49.9

always higher than the initial feed ratio of thioester (Figure 8). This means that under this reaction condition (120 °C, in bulk), cyclic thioester was preferentially recognized by the enzyme. This is an interesting result, since the overall enzymatic ring-opening polymerization of the cyclic thioester proceeded more slowly compared to the cyclic ester. These results also suggested that the rate-determining step for the ring-opening polymerization of the cyclic thioester was the nucleophilic attack by the mercapto group of the growing polymer species.

The water content of both the enzyme and the cyclic(hexanedithiol-sebacate) was determined by Karl Fischer coulometry. However, it is difficult to determine precisely the water content of lipase, which is responsible for the ring-opening reaction, using this method. An equilibrium exists between free water and loosely bound water at the lipase active site and other moieties of the lipase protein. Therefore, in this study, lipase was always dried under the same conditions before being used, and the cyclic monomers were also dried under the same conditions. After drying under vacuum over P_2O_5 at 25 °C for 2 h, the water contents of lipase CA and cyclic(hexanedithiol-sebacate) were 0.40 wt % and 0.51 wt %, respectively. In the case of the reaction shown in Figure 7, in which 20 mg of cyclic(hexanedithiol-sebacate) and 14 mg of lipase CA were used, this value corresponded to 8.79 μmol of water. Therefore, the content of water molecules in the lipase and cyclic(hexanedithiol-sebacate) (0.063 mmol) was calculated at approximately 13.9 mol % relative to the substrate. If all of this water is used for nucleophilic attack on the EAM, the initial conversion of the cyclic monomer should be 13.9 mol %. This value is slightly lower than that obtained experimentally (27 mol %). However, the relatively tightly bound water in lipase may be responsible for promoting the reaction by shifting the equilibrium. Such water cannot be measured by Karl Fischer coulometry.

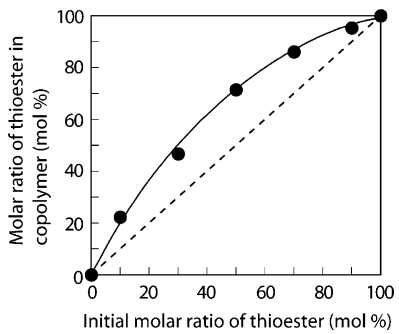


Figure 8. Composition of the copolymer. Reaction conditions: a mixture of cyclic thioester and cyclic ester was polymerized by lipase CA (70 wt %) in bulk at 120 °C with molecular sieves 4A at the top of the vial.

Properties of Poly(hexanedithiol-sebacate). The thermal properties of poly(hexanedithiol-sebacate) were evaluated and compared to those of the corresponding poly(hexanediol-sebacate) with the aim of investigating the difference between thioester and ester linkages in the polymer. The melting temperature (T_m), crystallization temperature (T_{cc}), and fusion enthalpy per unit (ΔH_u) were determined by DSC measurement. The fusion entropy per unit (ΔS_u) was calculated from the T_m and ΔH_u values. The glass transition temperature (T_g) could not

be obtained by DSC analysis, probably due to the high crystallinity of the polymer. The 5% weight loss decomposition temperature ($T_{d(5\%)}$) was also evaluated using TGA analysis. These results are summarized in Table 1. The T_m and T_{cc} values for poly(hexanedithiol-sebacate) were higher than those of the ester analogue poly(hexanediol-sebacate). Sulfur-containing polymers usually have a higher melting temperature when compared to the corresponding oxygen-containing polymer; this tendency was observed for the polythioesters examined in this study. Since the polythioester poly(hexanedithiol-sebacate) was found to have a lower ΔS_u (a parameter related to chain flexibility; a decrease in ΔS_u indicates that the polymer chain is more rigid[34]) than the corresponding polyester, the higher melting temperature of the polythioesters was concluded to be due to the greater rigidity of the thioester linkage.

Conclusion

A high molecular weight polythioester was prepared by enzymatic ring-opening polymerization of a cyclic thioester monomer. Poly(hexanedithiol-sebacate) with an M_w of 120 000 g/mol was prepared from cyclic(hexanedithiol-sebacate) monomer. In contrast to ring-opening polymerization of cyclic esters, the rate-determining step for ring-opening polymerization of the cyclic thioester was deacylation of the EAM by the propagating thiol chain. Poly(hexanedithiol-sebacate) was found to have a higher melting temperature than the corresponding ester analogue.

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