Enzymatic transformation of aliphatic polyesters into cyclic oligomers using enzyme packed column under continuous flow of supercritical carbon dioxide with toluene

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Abstract

The enzymatic degradation of chemically synthesized typical biodegradable plastics, such as poly(R,S-3-hydroxybutanoate), poly(ε-caprolactone) and poly(butylene adipate), into reactive cyclic oligomers under the continuous flow of supercritical or subcritical carbon dioxide (scCO₂) with toluene through an enzyme column was studied with reference to chemical recycling. It was confirmed that all the tested polymers were quantitatively transformed into the corresponding cyclic oligomers by passage through the column packed with immobilized lipase from Candida antarctica (Novozym 435) at 40 °C under the continuous flow of scCO₂ with some organic solvent. Compared to the pure organic solvents, by the addition of scCO₂ as a mobile phase degradation of the polymer was significantly promoted with respect to the reaction time, temperature and polymer concentration for complete transformation of the polyesters into oligomers through the enzyme column. This phenomenon is ascribed to the high diffusivity, low viscosity and better mass-transfer properties of scCO₂ as the reaction media. The degradation activity of the enzyme column was maintained for at least 6 months at 40 °C.

Keywords: Aliphatic polyesters; Chemical recycling; Lipases; Cyclic oligomers; Supercritical carbon dioxide fluids

1. Introduction

Biodegradable polymers, such as poly(lactic acid) and aliphatic polyesters, are now steadily growing in industrial production by both recyclable natural resources and petroleum resources. The production and processing of these polymers generally require a considerable amount of energy. In order to save the carbon resources and production energies, such biodegradable polymers should be recycled as much as possible. Among the polymer recyclings, chemical recycling with improved energy savings may be the ideal one. Chemical recycling of conventional and biodegradable plastics has been extensively studied; however, it generally requires much energy and catalysts, such as a base or an acid. Biodegradable plastics, such as polyesters and polycarbonates, are subjected to hydrolysis by the hydrolase enzyme as the chemical recycling [1–3]. We previously reported that certain kinds of biodegradable polymers are enzymatically degraded into repolymerizable cyclic oligomers. Chemically synthesized biodegradable polyesters, such as poly(R,S-3-hydroxybutanoate) [(R,S)-PHB] [4], poly(ε-caprolactone) (PCL) [3,5–7], poly(lactic acid) [8], poly(butylene succinate) [9] and poly(butylene adipate) (PBA) [9], and microbial polyhydroxyalkanolates [10] were degraded into cyclic oligomers in organic solvents using lipase (Scheme 1). This enzymatic degradation of aliphatic polyesters requires a large amount of enzymes for rapid transformation; therefore, a more efficient procedure is needed. In order to save enzymes, a continuous flow system was developed for the degradation of the polyesters. The enzymatic degradation of chemically synthesized polyester into a reactive cyclic oligomer under the continuous flow of toluene using an enzyme column has been reported [11,12]. However, both the flow and the batch methods required a large amount of organic solvent to degrade polymers into cyclic oligomers. Therefore, a more greener method was explored in order to reduce the amount of organic solvent used. Many of the solvents commonly used to dissolve polyesters, such as toluene and chloroform, are volatile organic compounds which may cause pollution and harm when released into the environment.
As a green solvent, supercritical and subcritical carbon dioxide (scCO2) and an ionic liquid [13–15] are currently being tested and evaluated with respect to the polymer synthesis and degradation by lipase. The scCO2 may have the potential as a solvent for polymer synthesis and recycling. Carbon dioxide can be readily recovered and recycled from the reaction mixture in a pure form. Some of the alternatives to traditional organic solvents include the use of scCO2. scCO2 is an adequate reaction medium because of its low toxicity, non-flammability, low viscosity, low surface tension and high diffusivity. Moreover, the availability of CO2 as byproduct of many industrial processes, its possible recycling, and easily accessible critical parameters account for its steadily increasing use. The lipase-catalyzed transesterifications of a diester and diol have been carried out in scCO2 by Chaudhary et al. and they reported that the molecular weight of the resulting polymer could be controlled by changing the pressure [16]. Hile and Pishko synthesized poly(lactide-co-glycolide) by a ring-opening precipitation polymerization in scCO2 using stannous octoate as the initiator [17]. More recently, the ring-opening polymerization of ε-caprolactone (ε-CL) initiated by dibutyltin dimethoxide was studied in scCO2 at 40 °C at a pressure of 210–215 bar by Stassin et al. [18]. The molecular weight of the produced PCL was about 20,000. Also, ring-opening polymerization of ε-CL in scCO2 was reported by Loeker et al. [19].

The lipase-catalyzed degradation into repolymerizable oligomers/monomers may establish a novel and sustainable polymer recycling system. However, the enzymatic transformation into repolymerizable oligomers requires a large amount of organic solvent which dissolves the polymers to facilitate the reaction and work-up procedures, such as enzyme recycling. Takamoto et al. reported that the lipase-catalyzed degradation of PCL in the presence of acetone produced a lower molecular weight oligomer of less than 500 in scCO2, which can be polymerized by the same catalyst [20,21]. Similar results were obtained by us such that PCL beads were transformed in scCO2 in the presence of a small amount of water and lipase to produce repolymerizable oligomers having an Me of about 500 [6]. The produced ε-CL oligomer was again polymerized with lipase from Candida antarctica (lipase CA) to yield a PCL having an Me greater than 80,000 [6].

In this report, the organic solvent was partially replaced by scCO2 for the transformation of biodegradable polyesters using an enzyme-packed column under a flow system.

2. Experimental

2.1. Materials and measurements

(R,S)-PHB (isotactic diad fraction [i] = 0.50, Mn = 110,000, Mw/Mn = 1.2) was synthesized by the anionic polymerization in bulk with potassium oleate/18-Crown-6 ether complex as an initiator [4]. PCL with Mn = 110,000 (Mw/Mn = 1.6) was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). PBA with Mn = 22,000 (Mw/Mn = 1.5) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Candida antarctica lipase immobilized on acrylic resin (lipase CA, Novozym 435 (triacylglycerol hydrolase + carboxyesterase) having 10,000 PLU/g (propyl laurate units: lipase activity based on ester synthesis) according to the supplier) was kindly supplied by Novozymes Japan Ltd (Chiba, Japan).

The weight-average (Mw) and number-average (Mn) molecular weights as well as molecular weight distribution (Mw/Mn) of the polymer and the produced oligomers were determined by a size exclusion chromatography (SEC) using SEC columns (GPC K-G + GPC K-804, Showa Denko Co., Ltd, Tokyo, Japan) with a refractive index detector (RI). For oligomers, the SEC column Shodex GPC K-801 (Showa Denko Co., Ltd, Tokyo, Japan) was used instead of K-804. Chloroform was used as the eluent. The SEC system was calibrated with polystyrene standards of a narrow molecular weight distribution. In the absence of commercially available polyester standards, in this report, the molecular weight was expressed as a value relative to the polystyrene standards. In the absence of commercially available polyester standards, in this report, the molecular weight was expressed as a value relative to the polystyrene standards.

1H NMR spectra were recorded with a JEOL Model JNM-LA300 (300 MHz) spectrometer (JEOL Ltd, Tokyo, Japan) and Varian NMR 300 (300 MHz) spectrometer (Varian, Inc., CA, USA) in CDCl3 with tetramethylsilane as an internal standard. The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was measured with Bruker Ultraflex mass spectrometer (Bruker Daltonics, Billerica, MA, USA). The spectrometer was equipped with a nitrogen laser. The detection was in the reflectron mode. 2,5-Dihydroxybenzoic acid (DHBA) was used as the matrix and positive ionization was used. The atmospheric pressure chemical ionization mass spectroscopy (APCI MS) was measured with a Finnigan LCQ LC/MS system.

2.2. Enzymatic transformation procedure using enzyme column under the continuous flow of scCO2 with toluene

The reaction system consisting of an enzyme-packed column placed in a column oven, scCO2 and solvent pumps (JASCO SCF-201, JASCO Ltd, Tokyo, Japan) and a
backpressure regulator (SCF-Bpg, JASCO Ltd, Tokyo, Japan) was used for the transformation of the polymer into the cyclic oligomer as shown in Fig. 1. The enzyme-packed column was prepared as follows. The immobilized lipase CA was soaked in toluene at 60 °C for 24 h to extract any soluble materials from the carrier particles. The enzyme was collected by filtration and dried under vacuum. The pretreated immobilized lipase CA (6.8 g) was packed in a 7.8 mm i.d. × 300 mm column. The enzyme column was equilibrated with toluene at 40 °C prior to use. The degradation of the polymer was carried out by injecting 1 mL of toluene solution containing 0.25–5% at a flow rate of 0.5–1.5 mL/min and a constant pressure between 9 and 20 MPa. The composition of scCO2 and toluene was regulated by the flow rate of scCO2 and toluene using dual pumps. The transformation into the cyclic oligomer was performed by the batch injection of 1.0 mL polymer solution through a 1.0 mL loop injector with subsequent elution by scCO2 and toluene at a constant flow rate. A typical degradation was carried out as follows. One milliliter of 0.25% (R,S)-PHB toluene solution was injected into the enzyme column at a flow rate of 0.5 mL/min with subsequent elution of a mixed solvent at a total flow rate of 0.5 mL/min using dual pumps at 0.4 mL/min of scCO2 and 0.1 mL/min of toluene at 15 MPa using a backpressure regulator. The enzyme column was kept at 40 °C. The eluents containing the degradation products were collected at the outlet of the backpressure regulator, and then the solvent was evaporated under slightly reduced pressure to quantitatively obtain waxy products. The composition and the chemical structure of the products were analyzed by 1H and 13C NMR, SEC, APCI MS and MALDI-TOF MS. The spectral data of cyclic 3-hydroxybutanoate oligomer, cyclic 6-hydroxyhexanoate dimer and cyclic butylene adipate (BA) dimer are shown to be representative. Cyclic 3-hydroxybutanoate dimer: 1H NMR (300 MHz; CDCl3): δ = 1.3 (–O–CH(CH3)–CH2–O–, 3H, m), 2.6 (–O–CH2(CH3)–CO–O–, 2H, m), 5.3 (–O–CH(CH3)–CH2–CO–O–, 1H, br), Cyclic 6-hydroxyhexanoate dimer: 1H NMR (300 MHz: CDCl3); δ = 1.35–1.75 (CH2 of C-3,4 and 5, 6H, m), δ = 2.38 (CH2 of C-2, 2H, t, J = 6.05 Hz), δ = 4.16 (CH3 of C-6, 2H, t, J = 5.63 Hz). Cyclic butylene adipate (BA) dimer: 1H NMR (300 MHz: CDCl3); δ = 1.68 (–O–CH2–CH2–CH2–CH2–O–, 4H, m), 1.73 (–CO–CH2–CH2–CH2–CO–, 4H, m), 2.34 (–CH2–CO–O–, 4H, m), 4.12 (–CH2–O–CO–, 4H, m).

3. Results and discussion

It was found that all the tested biodegradable aliphatic polyesters, such as (R,S)-PHB, PCL and PBA, were readily transformed into the corresponding cyclic oligomers by passage through the enzyme column packed with the immobilized lipase CA under the continuous flow of the mixed solvent of scCO2 and toluene at 40 °C and 15 MPa. The molecular structure of the produced 3-hydroxybutanoate, 6-hydroxyhexanoate and butylene adipate oligomers was analyzed by MALDI-TOF MS, SEC and 1H NMR with reference to our previous results [3,4,9,12]. From the 1H NMR, it was confirmed that no oligomer having an unsaturated terminal group (crotonate type) was detected in the produced oligomers when (R,S)-PHB was used. That is, no peak at δ = 7.0 (H–C(CH3)=CH–CO–O–) and 5.85 (H–C(CH3)=CH–CO–O–) was observed. Also, no peak at δ = 4.2 ascribed to terminal hydroxymethyl group (HO–CH) was detected [22]. Based on the 1H NMR and MALDI-TOF MS results, the produced 3-hydroxybutanoate oligomer mainly consisted of a cyclic structure. These results were in agreement with that obtained by the batch method as previously reported [4]. That is, by the batch process, the cyclic oligomer content was ca. 95% when atactic PHB was degraded by 500% lipase CA in 0.25% toluene solution at 40 °C for 72 h. The molecular structure of the 6-hydroxyhexanoate oligomer produced by the degradation of PCL was mainly a cyclic dimer with some higher molecular weight cyclic oligomers as measured by APCI MS, 1H NMR and SEC using the K-801 column [3,5–7]. Also, degradation products of PBA mainly consisted of a cyclic structure as analyzed by MALDI-TOF MS. We previously reported that cyclic oligomers of 6-hydroxyhexanoate and butylene adipate were readily polymerized by lipase or a conventional chemical catalyst to produce high-molecular weight polymers [3,9]. Therefore, these results indicated that using an enzyme column in combination with scCO2 might be an effective tool for the chemical recycling of biodegradable aliphatic polyesters.

3.1. The effects of the reaction pressure on enzymatic degradation

The solvent properties of supercritical fluids are dependent on both the pressure and temperature. Therefore, the effects of...
pressure on the transformation of the polyesters into the corresponding cyclic oligomers were analyzed at a pressure between 9 and 20 MPa at 40 °C. The degradation activity of lipase CA was maintained at least 6 months at 40 °C under these conditions.

Table 1 summarizes the effects of the reaction pressure on the molecular weight, molecular weight distribution and yield of the produced oligomers by passage through the enzyme column at 40 °C. That is, 1 mL of 0.25% polyester in toluene was injected into the enzyme column through a loop injector at a flow rate of 0.5 mL/min with subsequent elution mixing of scCO2 at a flow rate of 0.4 mL/min and toluene at 0.1 mL/min using dual pumps. It was found that (R,S)-PHB, PCL and PBA were all transformed almost quantitatively into cyclic oligomers having an \( M_n \) between 200 and 600 under these conditions. That is, the degradability of the polyesters into cyclic oligomers was not affected by the pressure between 9 and 20 MPa. The 3D SEC profiles of the degradation products from (R,S)-PHB are shown in Fig. 2. It was also confirmed that no unreacted polymer remained in the degradation mixture.

### Table 1

| Entry | Polymer  | Pressure (MPa) | Oligomer yield (%) | \( M_n \) | \( M_w/M_n \) |
|-------|----------|----------------|--------------------|----------|-------------|
| 1     | (R,S)-PHB| 9              | >99.9              | 540      | 1.45        |
| 2     | (R,S)-PHB| 15             | >99.9              | 580      | 1.62        |
| 3     | (R,S)-PHB| 20             | 99.4               | 510      | 1.92        |
| 4     | PCL      | 9              | >99.9              | 210      | 1.10        |
| 5     | PCL      | 15             | >99.9              | 220      | 1.10        |
| 6     | PCL      | 20             | >99.9              | 290      | 1.11        |
| 7     | PBA      | 9              | >99.9              | 320      | 1.31        |
| 8     | PBA      | 15             | >99.9              | 430      | 1.14        |
| 9     | PBA      | 20             | >99.9              | 370      | 1.21        |

3.2. The effects of scCO2 ratio in the mobile phase on the transformation of polyesters into the oligomers

In this study, the transformation was carried out using a mixed solvent of scCO2 and toluene as the mobile phase. Toluene acted as the solvent for the polymers in order to facilitate the reaction. Therefore, the effects of the ratio of scCO2 to toluene on the degradation were studied with respect to the required amount of organic solvent. The composition of scCO2 and toluene of the mobile phase was altered by each flow rate of scCO2 and toluene using the dual pumps, as shown in Fig. 1. That is, 1 mL of 0.25% polyester in toluene solution was injected into the enzyme column through a loop injector at a flow rate of 0.5 mL/min with subsequent elution of scCO2 and toluene at the total flow rate of 0.5 mL/min at 15 MPa and 40 °C.

It was found that the degradation of the polyesters was affected by the ratio of scCO2 and toluene in the mobile phase. That is, the rate of degradation of the polyesters into the cyclic oligomers was significantly facilitated with the increasing scCO2 content in the mobile phase. Fig. 3 shows the 3D SEC profiles of the degradation products of (R,S)-PHB (A) and PCL (B). It was found that the unreacted (R,S)-PHB and PCL polymers remained in the degradation mixtures when the pressure was varied from 9 to 20 MPa.

![Fig. 2. Effects of the pressure on the SEC profiles of 3-hydroxybutanoate oligomer produced by the degradation of (R,S)-PHB. The degradation conditions were the same as those in Fig. 1 except that reaction pressure was varied from 9 to 20 MPa.](image)

![Fig. 3. Effects of scCO2 content in the mobile phase on the transformation of polymer into the corresponding oligomers. (A) (R,S)-PHB, (B) PCL. The degradation conditions were the same as those in Fig. 1 except that the mobile phase composition of scCO2 and toluene was varied.](image)
scCO₂ content was lower than 60% for (R,S)-PHB and 40% for PCL, respectively, in the mobile phase. However, by increasing the scCO₂ content in the mobile phase, (R,S)-PHB and PCL were completely transformed into cyclic oligomers. These results showed that scCO₂ in toluene facilitated the degradation of the polyester. This is ascribed to the high diffusivity and better mass-transfer properties of scCO₂ compared to those of toluene. Details will be analyzed in the following reports. These results indicated that using scCO₂ allowed not only reducing the necessary amount of the organic solvent, but also enhancing the enzymatic degradability. On the other hand, PBA was completely transformed into the corresponding cyclic oligomers under the tested conditions due to the facile degradability with lipase CA.

### 3.3. The effects of flow rate on transformation of polyesters into oligomers

The reaction time of the polymer with an enzyme was determined by the flow rate through the enzyme column. Therefore, the effects of the flow rate on the molecular weight and molecular weight distribution of the producing oligomer were analyzed by changing the flow rate of the mobile phase. The flow rate was changed from 0.5 to 1.5 mL/min using a constant composition of 80% scCO₂ and 20% toluene in the mobile phase at 40 °C and 15 MPa. Table 2 summarizes the effects of the flow rate on the molecular weight, molecular weight distribution and yield of the oligomers. It was found that the flow rate of the mobile phase did not affect the transformation of PCL and PBA into the corresponding oligomers within these flow rate ranges. However, the molecular weight and molecular weight distribution of the produced oligomers from (R,S)-PHB were affected by the flow rate. Fig. 4 shows the 3D SEC profiles of the degradation products from (R,S)-PHB. The molecular weight distribution of the oligomer was increased by increasing the flow rate; however, no unreacted polymer remained at the highest tested flow rate of 1.5 mL/min. It was also found that the peak top molecular weight (Mₑ) of the oligomer slightly shifted to a higher molecular weight with the increasing flow rate. That is, Mₑ was increased from 480 to 1500. On the other hand, unreacted (R,S)-PHB remained when pure toluene was used without scCO₂ as the mobile phase at the flow rate of 1.5 mL/min. Also, it was regarded that scCO₂ facilitated the enzymatic degradation of the polyester. From these results, it was

| Entry | Polymer     | Flow rate (mL/min) | Oligomer yield (%) | Mₙ | Mₑ/Mₙ |
|-------|-------------|--------------------|--------------------|-----|-------|
| 1     | (R,S)-PHB   | 0.5                | 99.8               | 500 | 1.48  |
| 2     | (R,S)-PHB   | 1.0                | 99.2               | 660 | 1.67  |
| 3     | (R,S)-PHB   | 1.5                | 86.2               | 1010| 2.64  |
| 4     | PCL         | 0.5                | 98.4               | 210 | 1.10  |
| 5     | PCL         | 1.0                | 99.5               | 220 | 1.10  |
| 6     | PCL         | 1.5                | 99.8               | 220 | 1.10  |
| 7     | PBA         | 0.5                | >99.9              | 430 | 1.14  |
| 8     | PBA         | 1.0                | >99.9              | 400 | 1.23  |
| 9     | PBA         | 1.5                | >99.9              | 430 | 1.21  |

Fig. 4. Effects of the flow rate on the SEC profiles of produced 3-hydroxybutanoate oligomers by the transformation of (R,S)-PHB. The degradation conditions were the same as those in Fig. 1 except that flow rate was changed from 0.5 to 1.5 mL/min.

Fig. 5. Effects of the polymer concentration in the mobile phase on the transformation of polymer into the corresponding oligomers. (A) (R,S)-PHB, (B) PBA. The degradation conditions were the same as those in Fig. 1 except that polymer concentrations were varied from 1 to 5% in toluene.
suggested that the flow rate affected not only the transformation reaction of the polyester into the corresponding cyclic oligomer, but also changing the average molecular weight and the molecular weight distribution of the producing oligomer by the application of enzyme column using continuous flow of scCO$_2$ with toluene. Similar tendencies were observed for PCL and PBA, but the effect of the flow rate was small under the tested conditions due to their ready degradability by lipase CA.

3.4. The effects of the polymer concentration on the degradation of polyesters

The effects of the initial polymer concentration on the degradation of PHB and PBA were compared using a mobile phase consisting of 80% scCO$_2$ and 20% toluene solution at the flow rate of 0.5 mL/min at 40 °C and 15 MPa. The initial polymer concentration was changed from 1 to 5% polymer in toluene solution. The polymer concentration of higher than 5% caused a high viscosity thus interrupting the flow injection of the solution using a loop injector.

Fig. 5 shows the 3D SEC profiles of the degradation products of (R,S)-PHB and PBA as a function of the initial polymer concentration in toluene. It was found that the degradation occurred irrespective of the initial polymer concentration between 1 and 5% in toluene. That is, the average molecular weight, molecular weight distribution and oligomer yield were practically unchanged with the increasing polymer concentration from 1 to 5% in toluene. The same results were obtained for PCL under the initial polymer concentrations between 1 and 5% in toluene such that the polymer concentration in toluene did not affect the transformation of PCL.

When compared with the previous degradation results using pure toluene without scCO$_2$ as the mobile phase, the polymer transformation into the corresponding cyclic oligomer was significantly increased by utilizing scCO$_2$ as a component in the mobile phase. That is, when pure toluene was used as the mobile phase, 3% of the (R,S)-PHB solution was not completely degraded and a considerable amount of the unreacted polymer remained in the degradation products [11].

4. Conclusion

Aliphatic polyesters, such as (R,S)-PHB, PCL and PBA, were enzymatically transformed into the corresponding repolymerizable cyclic oligomers by passage through the enzyme column packed with immobilized lipase from C. antarctica under continuous flow of a mixed solvent of 80% scCO$_2$ and 20% toluene at 40 °C and 15 MPa. The reaction pressure and polymer concentration were not important factors for the transformation of the polymers into the corresponding oligomers. However, the scCO$_2$ content in the mobile phase and flow rate affected the reactions. The transformation rate was significantly increased with the increasing scCO$_2$ content up to 80% in toluene. This may be ascribed to the better mass-transfer properties of scCO$_2$ compared to those of toluene.

Acknowledgements

Immobilized lipase from C. antarctica (CA, Novozymes 435) was kindly supplied by Novozymes Japan Ltd (Chiba, Japan). This work was supported by a Grant-in-Aid for the 21st Century COE Program ‘KEIO Life Conjugate Chemistry’ and by a Grant-in-Aid for General Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

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