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Article

Enzymatic Synthesis and Crosslinking of Novel High Molecular Weight Polyepoxyricinoleate

Ayaki Kazariya and Shuichi Matsumura *

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

* Author to whom correspondence should be addressed; E-Mail: matumura@applc.keio.ac.jp; Tel.: +81-45-566-1582; Fax: +81-45-566-1582.

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Abstract: Methyl epoxyricinoleate was prepared in high yield by the lipase-catalyzed epoxidation of methyl ricinoleate with H_2O_2. A high molecular weight polyepoxyricinoleate (PER) with a maximum weight average molecular weight (M_w) of 272,000 was enzymatically prepared by the polycondensation of methyl epoxyricinoleate using immobilized lipase from *Burkholderia cepacia* (lipase PS-IM) in bulk at 80 °C for 5 d. PER showed good low temperature fluidability. PER was readily cured by maleic anhydride (MA) at 80 °C to produce a chloroform-insoluble PER-MA film. Both the glass transition temperature and Young's modulus increased with increasing MA content and PER M_w. In contrast, the elongation at break decreased with increasing MA content and PER M_w. Methyl epoxyricinoleate, PER and PER-MA showed biodegradability by activated sludge, and that of the PER-MA film decreased with increasing MA content.

Keywords: enzyme-catalyzed epoxidation; enzyme-catalyzed polymerization; biobased elastomer; green polymer; ricinoleic acid; epoxyricinoleic acid; polyepoxyricinoleate

1. Introduction

Aliphatic polyesters of high molecular weight were synthesized using highly effective transesterification catalysts and the vacuum technique as well as their chain extension reagents such as diisocyanates. However, a broader use of synthetic aliphatic polyesters is limited by their poor thermal

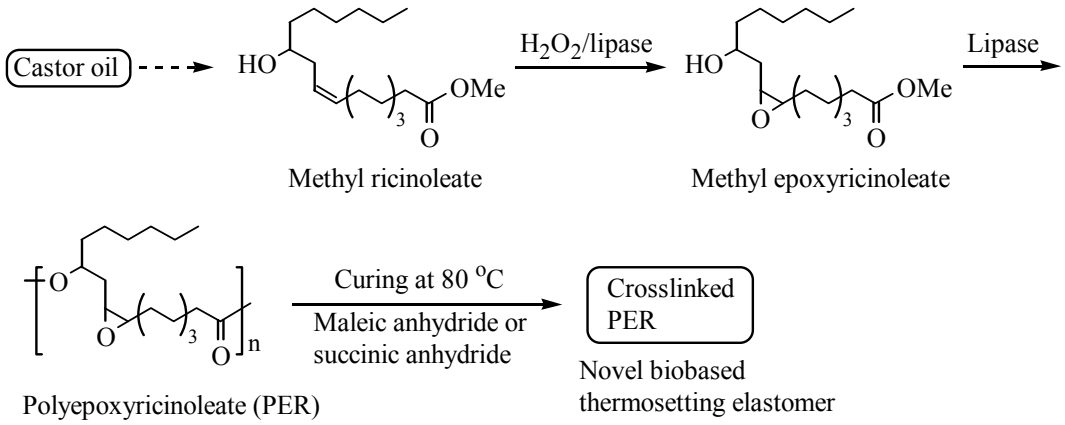
properties such as low melting temperatures [1]. In addition, the price of crude oil has increased in recent years, and the conservation of fossil resources is very important for establishing a sustainable society and reducing carbon dioxide emissions [2,3]. Biobased aliphatic polyesters produced from renewable biomass resources, such as poly(butylene succinate), are attractive with respect to replacing petroleum derived synthetic polyesters. Such green polymers should be produced by environmentally benign processes, e.g., enzymatic and solvent-free processes, and by avoiding the use and generation of hazardous materials such as metal catalyst.

Among the renewable raw materials for polymer production, some of the plant oils are the most promising candidates due to their characteristic molecular structure, non-volatility and biodegradability [4,5]. Edible plant oils are essentially used as a food source; however, the supply-demand balance for this market would break down if these oils were used for large-scale polymer production. Thus, the use of inedible resources has attracted much attention from the standpoint of global sustainability and the replacement of fossil resources in the polymer industries [6]. Inedible castor oil has attracted much attention as a building block for the preparation of functional materials due to its characteristic molecular structure and its abundant occurrence and relatively high purity comparable to a seed oil. Castor oil is obtained from the bean of the castor plant, *Ricinus communi* of the family Euphorbiaceae. Approximately 85–90% of the triglyceride-derived fatty acid in castor oil is 12-hydroxy-*cis*-9-octadecenoic acid (ricinoleic acid). We recently reported that ricinoleic acid can be enzymatically polymerized to yield high molecular weight polyricinoleate with a weight average molecular weight greater than 90,000. The produced polyricinoleate is a viscous liquid at room temperature with a glass transition temperature of −74.8 °C and is biodegraded by activated sludge [7]. Enzymatic polymerization has attracted attention in recent years as an environmentally benign process. Enzymes are renewable catalysts that exhibit high catalytic activities under mild conditions [8]. Thus, enzymatic polymerization is suitable for the synthesis of polymers with labile and reactive functional groups such as epoxy groups.

In order to increase the thermal properties of unsaturated polyesters, sulfur is widely used for the crosslinking of polymers. It has been suggested, however, that ill-smelling sulfur components are produced during degradation in the environment. Furthermore, the sulfide linkage is highly resistant to cleavage by microbes, which leads to resistance to biodegradation. It is known that acid anhydrides readily react with epoxy groups to produce hydrolyzable ester linkages, and many commercial epoxy adhesives and resins are produced using diacid anhydrides as curing agents, such as maleic anhydride and succinic anhydride [9,10]. Enzymatic epoxidation with lipase and hydrogen peroxide is an attractive oxidation process because of their mild and safe reaction, high conversion rate and easy removal of the catalyst. Furthermore, only water is produced in the reaction [11]. Miao *et al.* reported the enzymatic epoxidation of oleic acid followed by ring-opening polymerization with heating [12]. Enzymatic epoxidation is regarded as an environmentally benign process, and thus is effective for the synthesis of biobased derivatives.

In this report, a series of high molecular weight polyepoxyricinoleate (PER) polymers were prepared by enzymatic epoxidation with subsequent polycondensation of methyl ricinoleate using a lipase. The PER was then crosslinked using diacid anhydrides with the aim of creating novel biobased thermosetting resins (Scheme I). The thermal and mechanical properties, biodegradability of the PER and crosslinked PER polymers were measured.

Scheme I. Enzymatic synthesis and crosslinking of high molecular weight polyepoxyricinoleate.



2. Experimental Section

2.1. Materials

Methyl ricinoleate [methyl (*R*)-12-hydroxy-*cis*-9-octadecenoate] and molecular sieves 4A (MS4A) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The MS4A were dried at 150 °C for 2 h. Maleic anhydride (MA) was purchased from TCI Co., Inc. (Tokyo, Japan). Hydrogen peroxide (H_2O_2, 30 wt% aqueous solution) and succinic anhydride were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Immobilized lipase from *Candida antarctica* (CALB: Novozym 435, a lipase (B lipase) produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin and having 10,000 PLU/g (propyl laurate units: lipase activity based on ester synthesis)) was kindly supplied by Novozymes Japan, Ltd. (Chiba, Japan). Immobilized lipase from *Burkholderia cepacia* (lipase PS-IM Amano I: a lipase from *B. cepacia* immobilized on diatomaceous earth) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The enzyme was dried under vacuum (3 mmHg) over P_2O_5 at 25 °C for 2 h before use.

2.2. Measurements

The weight average (M_w) and number average (M_n) molecular weights, as well as the polydispersity (M_w/M_n) of the polymers, were determined by size exclusion chromatography (SEC) (Shodex K-G + K-805 columns, Showa Denko Co., Ltd., Tokyo, Japan) using a refractive index detector. Chloroform was used as the eluent at 1.0 mL·min^{-1}. The SEC system was calibrated with polystyrene standards having a narrow molecular weight distribution. The ^{1}H and ^{13}C-NMR spectra were recorded with an ECA-500 Fourier transform spectrometer (JEOL, Ltd., Tokyo, Japan) operating at 500 MHz and 125 MHz, respectively.

The glass transition temperature (T_g), melting temperature (T_m) and crystallization temperature (T_c) were determined by differential scanning calorimetry (DSC-60, Shimadzu, Kyoto, Japan). The measurements were made with a 10 mg sample on a DSC plate. The polymer samples were heated to 200 °C at the rate of 10 °C·min^{-1}, cooled to −100 °C at the rate of 20 °C·min^{-1} and then scanned with heating at the rate of 10 °C·min^{-1} from −100 to 200 °C.

The mechanical properties (Young's modulus, tensile strength and elongation at break) of the film samples were determined with an Autograph instrument (Shimadzu, Tokyo, Japan). The viscoelastic properties (storage elastic modulus) of the crosslinked polymers were measured by dynamic mechanical analysis (DMA) using an ARES viscoelastic measurement system (TA Instruments, Co., Ltd, New Castle, DE, USA). The temperature ramp tests were conducted in the range of −30–100 °C at the rate of 3 °C·min^{-1} at 1 Hz at a constant value of shear strain (3%). The T_g of the crosslinked polymers was also determined by DMA.

The biodegradability of methyl epoxyricinoleate, PER and PER-MA was evaluated using biochemical oxygen demand (BOD) measurements. The BOD was determined with a BOD tester (VELP Scientifica s.r.l., Usmate, MI, Italy) using the oxygen consumption method according to the modified MITI test [13]. The activated sludge was obtained from a municipal sewage plant in Yokohama City, Japan. The BOD biodegradation (BOD/Theoretical Oxygen Demand × 100) was measured for 34 d.

2.3. General Enzymatic Epoxidation Procedure

The general procedure for the enzymatic epoxidation of methyl ricinoleate was carried out in a screw-capped vial with MS4A placed at the top of the vial (vapor phase). Methyl ricinoleate (200 mg) and toluene (2 mL) were mixed in a vial at room temperature and then lipase (28 mg) was added followed by H_2O_2 (70 μL, 30 wt% aqueous solution), and the mixture was stirred at room temperature for 24 h. After the reaction, lipase was removed by filtration, and the filtrate was washed four times with water to remove any unreacted H_2O_2. The solvent was then evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (n-hexane/ethyl acetate = 4/1) to obtain methyl epoxyricinoleate in 77% yield as a colorless syrup. The molecular structure was analyzed by FT-IR, ^{1}H and ^{13}C-NMR spectroscopy.

FT-IR: 3449 (OH), 2928 (CH_2), 1740, 1171 (ester C=O), 831 (epoxy C-O-C) cm^{-1}.

^{1}H-NMR ($CDCH_3$): δ = 0.88 (*t*, *J* = 6.6 Hz, 3H, C*H*$_3$), 1.21–1.83 (*m*, 20H, C*H*$_2$), 2.31 (*t*, *J* = 7.5 Hz, 2H, 2-*H*2), 2.92 (*m*, 1H, 9-*H*), 3.14 (*m*, 1H, 10-*H*), 3.66 (*s*, 3H, COOC*H*$_3$), 3.87 (*m*, 1H, C*H*OH).

^{13}C-NMR ($CDCH_3$): δ = 14.08 (C-18 CH_3), 22.61 (C-17 CH_2), 24.88 (C-3 CH_2), 25.51/25.61 (C-14 CH_2), 26.43 (C-7 CH_2), 27.90/28.02 (C-8 CH_2), 29.01 (C-4 CH_2), 29.15 (C-15 CH_2), 29.26 (C-5 CH_2), 29.31 (C-6 CH_2), 31.81 (C-16), 34.06 (C-2 CH_2), 34.72/35.17 (C-11 CH_2), 37.46/37.77(C-13 CH_2), 51.47 (COO*C*H$_3$), 54.43/55.47 (C-10 HC-O), 56.31/57.10 (C-9 HC-O), 70.20/70.96 (C-12 CH),174.3 (C-1 C-O).

2.4. General Enzymatic Polymerization Procedure

The general procedure for the enzymatic polymerization of methyl epoxyricinoleate was carried out in a screw-capped vial with MS4A placed at the top of the vial (vapor phase) to absorb byproducts, such as water or methanol [14–17]. The preparation of PER with a M_w of 272,000 is described as a typical example. Methyl epoxyricinoleate (50 mg, 0.16 mmol) was polymerized in the presence of lipase PS-IM (50 mg) under a nitrogen atmosphere at 80 °C for 7 d. The polymerization mixture was dissolved in hot chloroform (20 mL), and the insoluble enzyme was removed by filtration. The solvent was next evaporated to obtain the polymer as a colorless viscous syrup, which was then purified by

reprecipitation using chloroform (good solvent)–methanol (poor solvent) to remove unreacted monomers. The M_w and the M_w/M_n of the polymer were determined using SEC. The molecular structure was analyzed using FT-IR, ^{1}H and ^{13}C-NMR spectroscopy. The spectral data for PER with a M_w of 272,000 is shown as an example.

FT-IR: 2930 (CH_2), 1732, 1175 (ester C=O), 835 (epoxy C-O-C) cm^{-1}.

^{1}H-NMR: δ = 0.88 (*t*, *J* = 6.6 Hz, 3H, C*H*$_3$), 1.21–1.80 (*m*, 20H, C*H*$_2$), 2.30 (*t*, *J* = 7.5 Hz, 2H, 2-*H*2), 2.87 (*m*, 1H, 9-*H*), 2.96 (*m*, 1H, 10-*H*), 5.06 (*m*, 1H, *H*C-O).

^{13}C-NMR: δ = 14.08 (C-18 CH_3), 22.57 (C-17 CH_2), 25.01 (C-3 CH_2), 25.24/25.37 (C-14 CH_2), 26.61 (C-7 CH_2), 27.96/28.02 (C-8 CH_2), 29.06 (C-4 CH_2), 29.11 (C-15 CH_2), 29.27 (C-5 CH_2), 29.39 (C-6 CH_2), 31.72 (C-16 CH_2), 32.72/32.79 (C-11 CH_2), 34.17/34.37 (C-13 CH_2), 34.60 (C-2 CH_2), 53.70/53.85 (C-10 HC-O), 56.15/56.79 (C-9 HC-O), 72.15/72.19 (C-12 CH), 173.5 (C-1 C-O).

2.5. General Crosslinking Procedure for PER

The crosslinking of PER with a M_w of 186,000 is described as a typical example. PER (200 mg) and MA (62.8 mg, 0.64 mmol) as a crosslinking agent were dissolved in hot chloroform (5 mL). The obtained viscous solution was poured onto a Teflon sheet and then dried in air for 12 h to vaporize the chloroform. After vaporizing the chloroform, the viscous liquid reaction mixture was held between the Teflon sheets with a thickness of 0.3 mm, and then cured at 80 °C for 30 min using a hot press machine. The viscous PER was readily cured by MA at 80 °C for 30 min to form a maleic anhydride-crosslinked PER (PER-MA) film with a thickness of 0.3 mm.

The gel fraction of the crosslinked PER was determined from the weight remaining after soaking of the sample in chloroform using the following equation:

$$\text{Gel fraction (\%)} = (W_g/W_0) \times 100$$

where W_0 is the weight of the (dry) crosslinked PER before soaking and W_g is the weight of the crosslinked PER remaining (dry gel component) after soaking in chloroform at room temperature for 48 h. The molecular structure was analyzed using FT-IR spectroscopy.

FT-IR: 2923 (CH_2), 1731, 1176 (ester C=O), 1647 (C=C) cm^{-1}

In a similar procedure, succinic anhydride-crosslinked PER (PER-SA) was prepared by the reaction of PER and succinic anhydride.

3. Results and Discussion

3.1. Lipase-Catalyzed Epoxidation of Methyl Ricinoleate

Various kinds of lipases were screened with respect to epoxidation of methyl ricinoleate using a 14 wt% enzyme (relative value to methyl ricinoleate) at room temperature for 24 h. Among the tested lipases, CALB showed the highest catalytic activity. In the absence of CALB or in the presence of thermally deactivated CALB, no significant epoxidation of methyl ricinoleate was observed at room temperature. In addition, in the absence of H_2O_2, no significant epoxidation was observed. These results indicate that CALB is an active catalyst for epoxidation in the presence of H_2O_2.

The effect of the epoxidation conditions, such as the amount of H_2O_2 and reaction time, are shown in Figures 1 and 2, respectively. The conversion of methyl ricinoleate to methyl epoxyricinoleate increased gradually with increasing amount of H_2O_2 from 5 to 13 wt% (relative value to methyl ricinoleate). The optimization of the H_2O_2 content is important for reducing byproducts (ricinoleic acid) and for improving the safety of the reaction with respect to the amount of residual peroxide after completion of the reaction. A high content of H_2O_2 may produce surplus peroxyricinoleic acid and might also deactivate CALB. Thus, the best results were obtained at 11 wt% H_2O_2 relative to methyl ricinoleate, where the conversion approached 100%. On the other hand, the conversion of methyl ricinoleate increased quickly with reaction time from 0 to 6 h, and then it increased gradually from 6 to 24 h and reached 100% after 24 h. Lipase-catalyzed epoxidation of unsaturated fatty acids has been reported by some authors [18–20]. Based on these reports, Figure 3 shows the proposed mechanism of lipase-catalyzed epoxidation of methyl ricinoleate using H_2O_2. As the first step, an acyl-enzyme intermediate is formed by the reaction of a serine hydroxy group, followed by reaction with H_2O_2 in the presence of the imidazole group of a hystidine unit of CALB to produce peroxyricinoleic acid. As the second step, the π-electrons of the double bond of methyl ricinoleate are attacked by peroxyricinoleic acid to produce methyl epoxyricinoleate. Simultaneously, ricinoleic acid is produced as a byproduct and converted to peroxyricinoleic acid by H_2O_2 and CALB via an acyl-enzyme intermediate, and then the double bond is attacked by the peroxyricinoleic acid to form methyl epoxyricinoleate again. This series of steps continues successively to complete the reaction.

The obtained methyl epoxyricinoleate exhibited characteristic FT-IR absorptions at 1,740 and 1,171 cm^{-1} corresponding to the ester carbonyl stretching bands, and at 831 cm^{-1} corresponding to the epoxy stretching band. The presence of the epoxy group was also confirmed by ^{1}H-NMR spectroscopy (δ = 2.92 and 3.14).

Figure 1. Conversion of methyl ricinoleate to methyl epoxyricinoleate as a function of H_2O_2 content. Reaction conditions: Methyl ricinoleate (200 mg, 0.64 mmol) was epoxidized by aqueous H_2O_2 and CALB (28 mg, 14 wt% relative to methyl ricinoleate) in toluene (2 mL) at room temperature.

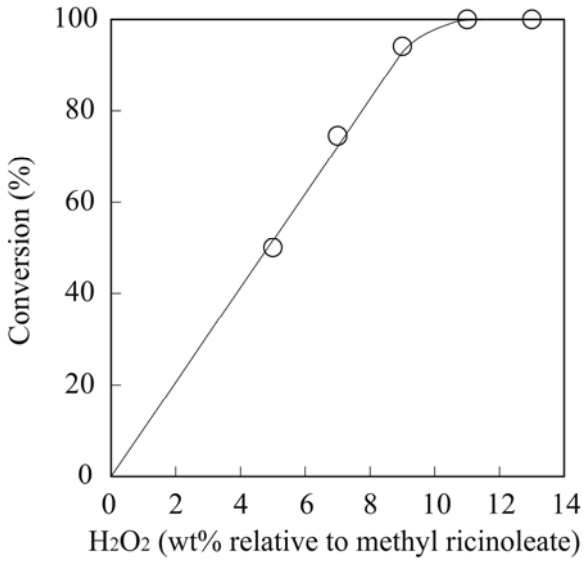


Figure 2. Time course of the conversion of methyl ricinoleate to methyl epoxyricinoleate. Reaction conditions: Methyl ricinoleate (200 mg, 0.64 mmol) was epoxidized by aqueous H_2O_2 (11 wt% relative to methyl ricinoleate) and CALB (28 mg, 14 wt% relative to methyl ricinoleate) in toluene (2 mL) at room temperature.

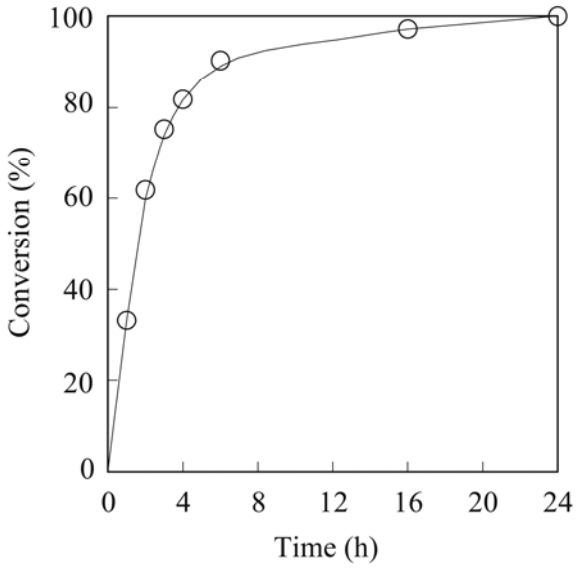
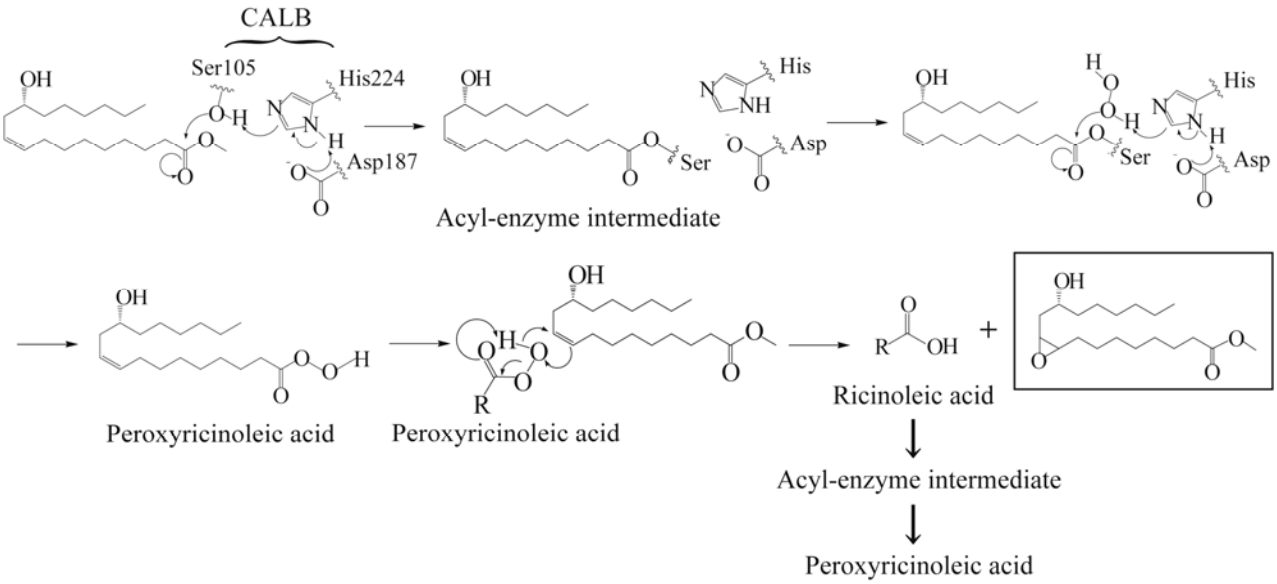


Figure 3. Proposed mechanism for the lipase-catalyzed epoxidation of methyl ricinoleate by H_2O_2.



3.2. Lipase-Catalyzed Polymerization of Methyl Epoxyricinoleate

Various kinds of lipases were screened with respect to polymerization of methyl epoxyricinoleate using a 100 wt% enzyme (relative value to methyl epoxyricinoleate) at 70 °C for 7 d. The enzyme was dried over P_2O_5 under vacuum before use. The results are summarized in Table 1. It was found that the immobilized lipase from *Burkholderia cepacia* (PS-IM) showed the highest catalytic activity among the tested lipases. No significant polymerization of methyl epoxyricinoleate was observed without lipase under these conditions, indicating the responsibility of the lipase in the polymerization.

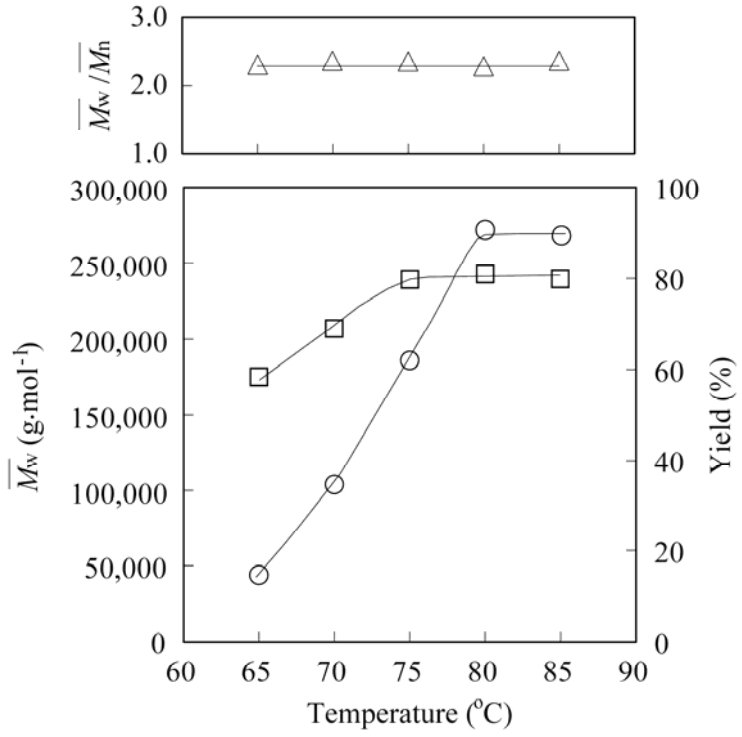
Table 1. Polymerization of methyl epoxyricinoleate using various lipases at 70 °C for 7 d.

Entry	Lipase [a)]	M_w (g mol^{-1})	M_w/M_n
1	*Candida antarctica* (CALB)	68,700	2.2
2	*Candida rugosa* (lipase CR)	<800	1.1
3	*Burkholderia cepacia* (lipase PS-IM)	99,100	2.4
4	*Pseudomonas fluorescens* (lipase AK)	24,800	1.8
5	Thermally diactivated lipase PS-IM	<800	1.1
6	Blank	<800	1.1

[a)] Lipase concentration: 100 wt% relative to methyl epoxyricinoleate.

The M_w of the polymer was influenced by the reaction temperature, as shown in Figure 4. The M_w values of PER increased quickly with increasing reaction temperature from 65 to 80 °C, and were almost constant at around 270,000 g·mol^{-1} at a temperature between 80 and 85 °C. Furthermore, the polydispersity of the polymer was also constant at around 2.3. In the absence of lipase PS-IM, or in the presence of thermally deactivated lipase PS-IM, no significant polymerization was observed at 80 °C. These results indicate that lipase PS-IM is an active catalyst for the polymerization process.

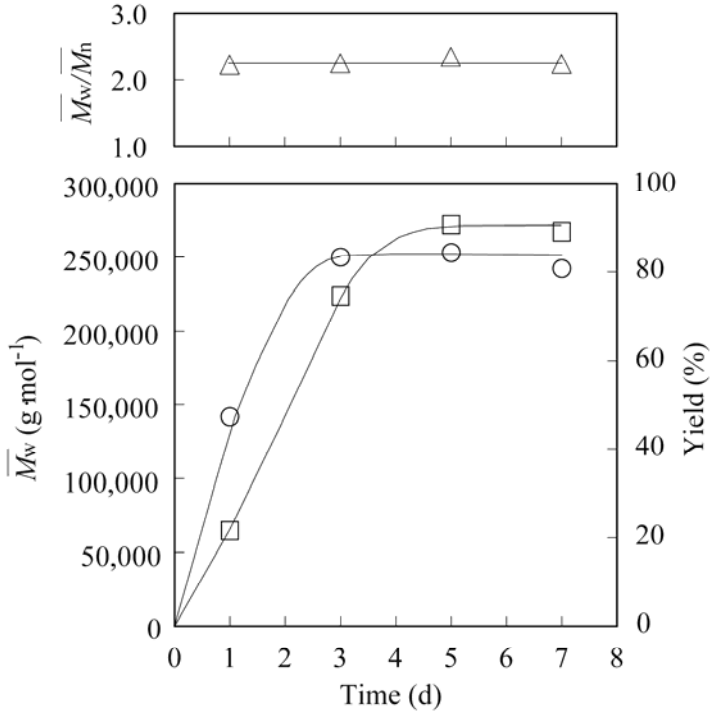
Figure 4. M_w (○), M_w/M_n (△) and yield (□) of polyepoxyricinoleate (PER) as a function of polymerization temperature. Reaction conditions: Methyl epoxyricinoleate (50 mg, 0.16 mmol) was polymerized by lipase PS-IM (100 wt% relative to methyl epoxyricinoleate) in bulk for 7 d.



It was also found that the M_w of the polymer was influenced by the reaction time. Figure 5 shows the PER M_w produced by enzymatic polymerization as a function of time using 100 wt% lipase PS-IM at 80 °C in bulk with MS4A. The M_w values of the PER increased quickly with the reaction time from 1 to 5 d, and then remained almost constant at around 270,000 g·mol^{-1}. The polydispersity of the

polymer was also constant at around 2.3. Thus, the best polymerization conditions were determined to be 80 °C for 5 d. Although lipase PS-IM was immobilized on diatomaceous earth, a relatively large amount of immobilized lipase was necessary for rapid polycondensation. Based on these results, it was concluded that a high molecular weight PER was produced using lipase PS-IM at 80 °C for 5 d.

Figure 5. M_w (○), M_w/M_n (△) and yield (□) of PER as a function of polymerization time. Reaction conditions: Methyl epoxyricinoleate (50 mg, 0.16 mmol) was polymerized by lipase PS-IM (100 wt% relative to methyl epoxyricinoleate) in bulk at 80 °C.



The obtained PER with a M_w of 272,000 showed a typical FT-IR absorption at 1731 and 1175 cm^{-1} corresponding to the ester carbonyl stretching bands, and at 835 cm^{-1} corresponding to the epoxy stretching band. The presence of the epoxy group was also confirmed by ^{1}H-NMR spectroscopy (δ = 2.87 and 2.96). Essentially no peak for the terminal methyne proton at δ = 3.66 appeared in the ^{1}H-NMR spectrum, suggesting a high polymerization degree.

3.3. Crosslinking of PER

The crosslinking features of PER were investigated. PER (200 mg) with a M_w of 186,000 and MA (62.8 mg, 0.64 mmol) as a crosslinking agent were dissolved in chloroform (5 mL). The obtained viscous solution was poured onto a Teflon sheet and then dried in air for 12 h to vaporize the chloroform. After vaporizing the chloroform, the residual film was cured at 80 °C for 30 min using a hot press machine. The viscous PER was readily cured by MA at 80 °C for 30 min to form a maleic anhydride-crosslinked PER (PER-MA) film with a thickness of 0.3 mm. The PER-MA sheet was soaked and swelled in chloroform in order to dissolve the uncrosslinked fraction. As determined by the weight percentage of the dissolved fraction in the chloroform, the gel fraction of the PER-MA was 99.2%. The film had a smooth surface. The total transmission of the film was about 91% and the

HAZE was around 20%. The FT-IR absorption peak at 1,647 cm^{-1}, corresponding to the C=C stretching band of the crosslinker in the PER-MA sample, was observed after curing of the PER, and the peak at 835 cm^{-1} corresponding to the epoxy stretching band disappeared. This result indicated that the crosslinking occurred at the epoxy group via a ring-opening reaction with MA.

3.4. Thermal Properties

The thermal properties of the PER, PER-MA and PER-SA polymers were analyzed at the second heating at a rate of 10 °C·min^{-1}. It was found that a single T_g peak was observed for each polymer sample. The results are summarized in Table 2. The PER with a M_w of 186,000 was a viscous liquid at room temperature and showed no T_m or T_c, but showed a T_g at −49.4 °C. It was found that PER showed good low temperature fluidability and may have applications in the various fields such as thickners. The corresponding PER-MA (PER M_w of 186,000, MA content of 100 mol%) also showed no T_m or T_c but showed a T_g of 2.2 °C. The T_g increased significantly after crosslinking, probably due to the restriction of the polymer chains. Furthermore, the T_g of PER-MA increased gradually with increasing MA content. These results suggest that MA reacted as a crosslinker, and the crosslinking degree was dependent on the molar ratio of PER and MA. On the other hand, the T_g of PER-MA increased gradually with increasing PER M_w. As the PER M_w increased, the number of free chains decreased, and thus the structure of PER-MA tended to form a perfect network. In addition, the PER-SA sheet (PER M_w of 186,000, SA content of 100 mol%) showed a lower T_g than the corresponding PER-MA sheet (PER M_w of 186,000, MA content of 100 mol%). The PER-SA has no double bonds in the crosslinker moiety, so the restriction of the polymer chain is weaker than that of the PER-MA.

Table 2. The thermal and mechanical properties of PER- maleic anhydride (MA) and PER-succinic anhydride (SA) films with different crosslinker contents and PER M_w.

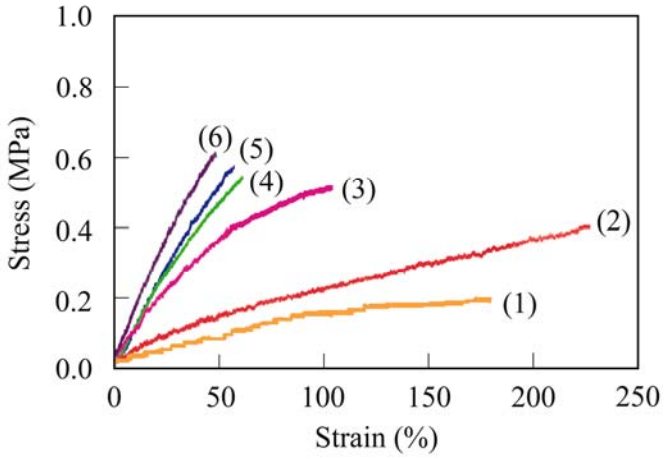
	Crosslinker Content (mol%)	**M_w of PER (g·mol^{-1})**	**T_g (°C)**	**Young's Modulus (MPa)**	**Tensile Strength (MPa)**	**Elongation at Break (%)**	**G' (25 °C) (MPa)**	**G' (T_g +50 °C) (MPa)**
PER-MA	20	186,000	−17.8 [a)]	0.408	0.404	220	-	-
	40	186,000	−12.4 [a)]	0.850	0.507	101	-	-
	60	186,000	−8.8 [a)]	1.11	0.543	60.1	-	-
	80	186,000	−2.9 [a)]	1.25	0.573	56.4	-	-
	100	186,000	2.2 [a)]	1.38	0.621	48.8	-	-
	100	94,000	−5.5 [b)]	0.638	0.715	171	0.890	0.744
	100	147,000	5.1 [b)]	1.08	0.680	102	3.89	1.24
	100	172,000	15.0 [b)]	1.30	0.693	64.0	5.98	2.10
	100	224,000	22.0 [b)]	1.49	0.675	47.7	13.8	4.76
PER-SA	100	186,000	−4.2 [a)]	0.156	0.220	178	-	-

[a)] determined by DSC at second heating (heating rate: 10 °C·min^{-1}); [b)] determined by DMA (heating rate: 3.0 °C·min^{-1}).

3.4. Mechanical Properties

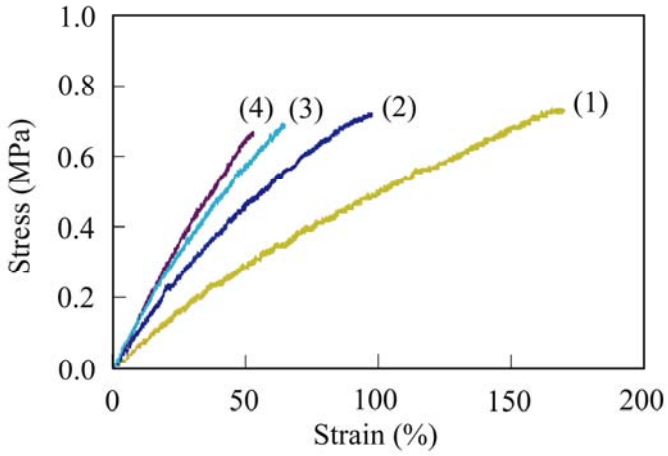
The mechanical properties of the PER-MA and PER-SA films using dumbbell specimens with a thickness of 0.3 mm were measured by tensile tests at room temperature. The stress-strain curves for PER-SA and PER-MA films with varying degrees of crosslinking were obtained, and the Young's modulus, tensile strength and elongation at break of the PER-MA and PER-SA films were calculated. The results are shown in Table 2 and Figure 6. It was found that these mechanical properties were relatively low and highly dependent on the MA content. With increasing MA content, the Young's modulus increased, while the elongation decreased. No yield points for the PER-MA films were observed. The Young's modulus increased in a linear fashion suggesting that MA reacted as a crosslinker depending on the molar ratio of PER and MA. On the other hand, the PER-SA film made from PER with a M_w of 186,000 and an SA content of 100 mol% showed a lower Young's modulus and tensile strength, while a higher elongation was observed when compared to those of the corresponding PER-MA film (PER M_w of 186,000, MA content of 100 mol%). As stated before, because the PER-SA film has no double bonds in the crosslinker moiety, the restriction of the polymer chains is weaker than it is in the corresponding PER-MA film. It was also found that the Young's modulus increased with increasing the M_w of the parent PER as shown in Figure 7.

Figure 6. Stress-strain curves of PER-SA (PER M_w of 186,000, SA content of 100 mol%) and PER-MA (M_w of PER 186,000) films with different MA content with a thickness of 0.3 mm. (1) SA content of 100 mol%, (2) MA content of 20 mol%, (3) MA content of 40 mol%, (4) MA content of 60 mol%, (5) MA content of 80 mol%, (6) MA content of 100 mol%.



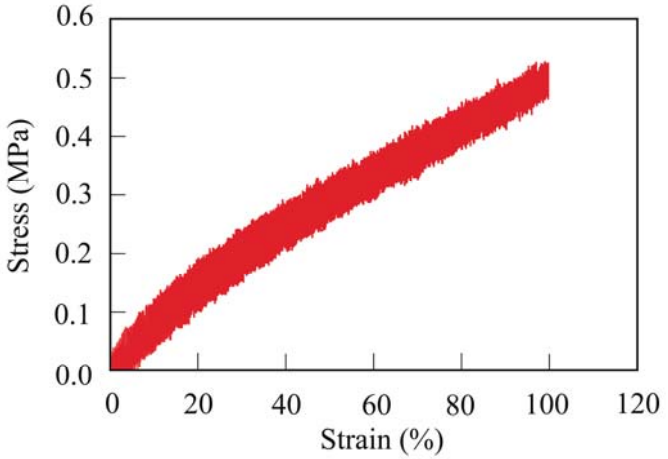
The storage elastic modulus (G') of the PER-MA films with a thickness of 1.0 mm was measured using dynamic viscoelastic measurements. The results are shown in Table 2. It was found that these properties were highly dependent on the PER M_w. With increasing PER M_w, the Young's modulus increased, while elongation decreased. The G' of the PER-MA films at 25 °C also increased with increasing PER M_w. The PER-MA prepared using PER with a M_w of 94,000 was almost rubbery, while the PER-MA prepared from a higher M_w PER was not fully rubbery, but almost glassy at 25 °C. In addition, the G' at T_g+50 °C increased with increasing PER M_w. This result is due to decreasing number of free chains with increasing PER M_w, and the increasing number of polymer chains that are tangled.

Figure 7. Stress-strain curves of PER-MA (MA content of 100 %) with different M_w of PER. (1) M_w of 94,000, (2) M_w of 147,000, (3) M_w of 174,000, (4) M_w of 224,000.



The cyclic stress-strain curves of the PER-MA films with a thickness of 0.3 mm were obtained from cyclic loading tests. In this test, the sample films were cyclically loaded and unloaded five times to the maximum elongation of 100% based on the initial length of the test film. As a typical example, the stress-strain curve of the PER-MA sheet formed from PER with a M_w of 94,000 and an MA content 100 mol% in the cyclic loading test is shown in Figure 8. The cyclic tensile test revealed that the strain recovery of the PER-MA film was nearly 100%, indicating that it has an elastomer's ability to recover from deformation. Thus, the polymer is suitable for applications with a mechanically dynamic environment.

Figure 8. Stress-strain curves of PER-MA (PER M_w of 94,000, MA content of 100 mol%) in the hysteresis experiment.



3.5. Biodegradability

The biodegradability of methyl epoxyricinoleate, PER with a M_w of 186,000, PER-MA films with varying MA content and the PER-SA film with an SA content of 100 mol% evaluated using activated sludge according to the BOD test is shown in Figures 9 and 10. Aniline was used as a reference for the biodegradation test. Figure 9 shows that the biodegradation took place gradually, and that the

BOD-biodegradability of methyl epoxyricinoleate and PER were over 60 % after 28 d and 34 d, respectively. These results indicate good biodegradability of methyl epoxyricinoleate and PER. From Figure 10, it can be seen that the biodegradation of the crosslinked PER took place slowly, and that the BOD-biodegradability of the PER-MA films decreased with increasing MA content. It appears that the ester bonds in the crosslinker moiety are cleaved first, and then the ester bonds and the methylene chains of polymer are biodegraded slowly. The larger the MA content, the longer the time required to cleave the ester bonds in the crosslinker moiety, and thus a reduction in the BOD-biodegradability was observed. It was also found that the PER-SA film (PER M_w of 186,000, SA content of 100 mol%) was biodegraded slightly faster than that of the PER-MA film (PER M_w of 186,000, MA content of 100 mol%). The PER-SA film has no double bonds in the crosslinker moiety and is much more flexible than the PER-MA film; thus, after cleaving the ester bonds at the crosslinker moiety, the biodegradation occurs more quickly.

Figure 9. Time course of the BOD biodegradation of methyl epoxyricinoleate and PER.

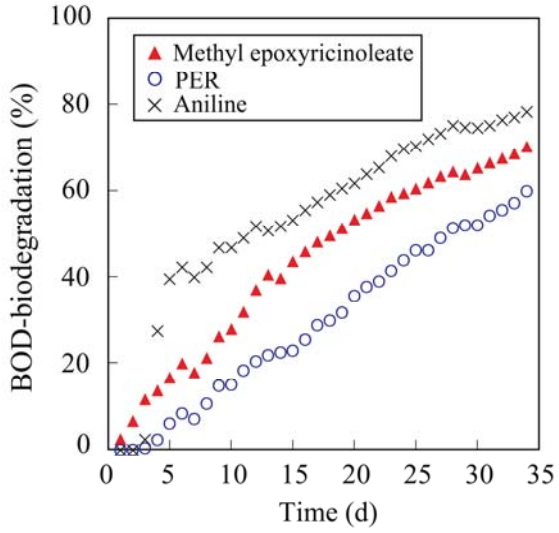
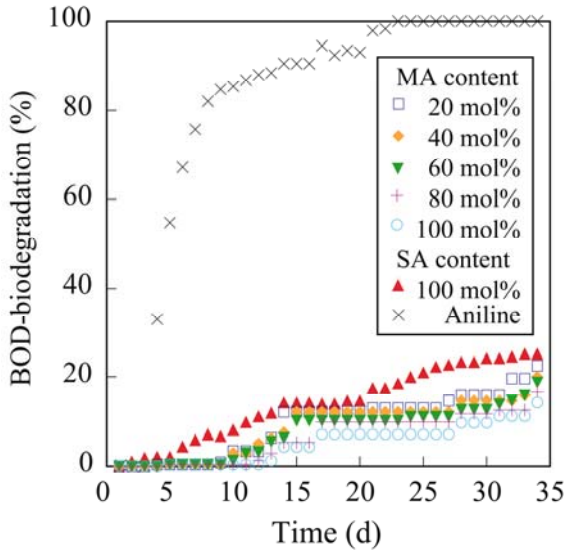


Figure 10. Time course of the BOD biodegradation of PER-SA and PER-MA films with different MA content.



4. Conclusions

Methyl ricinoleate was epoxidized by CALB and H_2O_2 to produce methyl epoxyricinoleate in high yield. Methyl epoxyricinoleate was polymerized by lipase PS-IM to produce a high molecular weight PER with a maximum M_w of 272,000. PER showed good low temperature fluidability and good biodegradability. Thus, PER has potentials for applications as biobased and biodegradable additives for low temperature. PER was readily cured by heating with MA without any catalyst to produce PER-MA. As analyzed by DSC and DMA, the T_g increased with increasing MA content (crosslinking degree) and PER M_w, as did the Young's modulus. In contrast, the elongation at break decreased with increasing MA content and PER M_w. The strain recovery of PER-MA was nearly 100%, indicating that it has an elastomeric ability to recover from deformation. Methyl epoxyricinoleate, PER, PER-MA and PER-SA were biodegraded by activated sludge. The biodegradability of the PER-MA films decreased with increasing MA content. Based on these results, PER-MA films have potentials for applications as biobased thermosetting resins in the field of environmentally benign polymeric materials.

Acknowledgments

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