Novel lipase-catalyzed ring-opening copolymerization of oxiranes and dicarboxylic anhydride forming polyesters bearing carboxyl groups and their physicochemical properties and biodegradability

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SUMMARY: Oxiranes, such as benzyl glycidate and glycidyl phenyl ether, were copolymerized with dicarboxylic anhydride by lipase at a temperature between 60 and 80 °C to yield the corresponding polyesters bearing carboxyl or phenyl groups. Bulk polymerization, especially at 80 °C, and preferably using porcine pancreatic lipase, gave biodegradable polyesters with a molecular weight of greater than 10000. Poly(sodium carboxylate)s containing ester linkages in the backbone prepared in this study were readily biodegradable by the activated sludge and exhibited a calcium ion sequestration capacity.

Introduction

High-molecular weight polycarboxylates have been considered attractive as water-soluble functional polymers both in the industrial and biomedical fields. However, they are generally highly resistant to biodegradation which is an important criterion in large scale applications. Environmentally acceptable polycarboxylates are particularly needed, and great efforts have been made to develop such biodegradable polymers. Among the high-molecular weight polycarboxylates, only a few polymers, which contain ester or amide linkages in the backbone, such as poly(malic acid) [1,2], poly(γ-glutamic acid) [3,4] and poly(aspartic acid) [5-9], are biodegradable [10,11]. Poly(malic acid) is a biodegradable and bioadsorbable water-soluble polyester having modifiable pendant carboxylic groups. Recently, in the pharmaceutical fields, this polymer attracted attention as a polymer carrier which is able to covalently attach drug units and targeting agents [12-15] and can also be used as a biodegradable raw material for the chemical industries such as detergent builders and chelating agents [1]. The chemical method for the preparation of poly(β-malic acid), first reported by Vert and Lenz, showed that the ring-opening polymerization of benzyl β-malolactonate is involved [16-19]. Copolymerization by the combination of benzyl β-malolactonate and other lactones were also reported [20-22]. However, the preparation of cyclic lactones bearing functional groups needs multi-step reactions and the overall yields are generally low. A convenient way to produce functional polyesters is using

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oxiranes bearing the selected functional groups and dicarboxylic anhydrides, because oxiranes bearing the functional groups are synthesized with relative ease compared to the lactones such as benzyl β-malolactonate[1,12,18,19,23,24]. However, the conventional method for the ring-opening copolymerization of oxiranes with acid anhydride requires extremely pure monomers and anhydrous conditions as well as an organometallic catalyst [25,26], which must be completely removed before use in medicinal applications. To avoid these difficult restrictions for the ring-opening polymerization by chemical methods, enzyme-catalyzed polymerization may be one of the feasible methods to obtain such functional polyesters [27-29]. However, the enzymatic ring-opening polymerization method was restricted to cyclic lactones [30-35], and the ring-opening copolymerization of oxiranes with acid anhydrides using an enzyme, so far, has not been reported except for our recent communications [36].

In this report, the preparation of functional polyesters by the novel enzymatic ring-opening copolymerization of dicarboxylic anhydride with oxiranes bearing carboxyl and phenyl groups was studied with respect to the reaction conditions and the origin of the enzyme. Their hydrolytic stability, calcium ion sequestration capacity and biodegradability were evaluated.

Experimental part

Materials and measurements

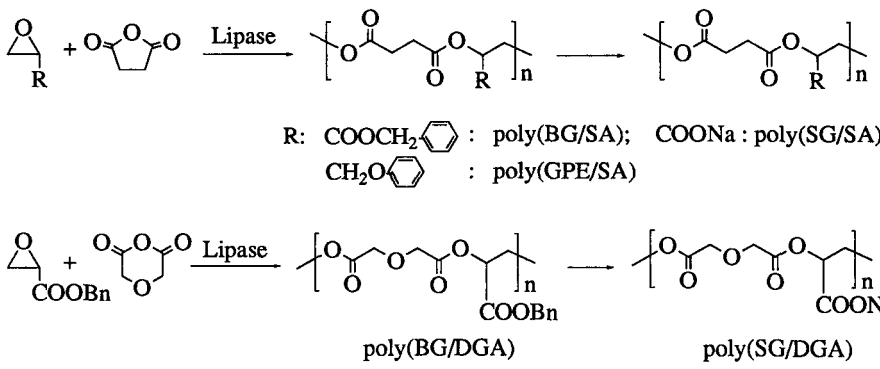
Benzyl glycidate (BG) was prepared by the epoxidation of benzyl acrylate with 3-chloroperoxybenzoic acid according to the method of Muggee et al.[37]. BG was further purified by silica gel column chromatography using hexane-acetone (9:1, v/v) as an eluent. Glycidyl phenyl ether (1,2-epoxy-3-phenoxypropane: GPE) and diglycolic anhydride (DGA) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). GPE was distilled under reduced pressure before use. Porcine pancreatic lipase (PPL, 190 unit/mg protein, using olive oil, according to the supplier), purified porcine pancreatic lipase (30000 unit/mg protein, using olive oil, according to the supplier) and lipase from *Candida cylindracea* (CC, 943 unit/mg, using olive oil, according to the supplier) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Lipase PS was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Novozym 435 (triacylglycerol hydrolase + carboxylesterase) having 7000 PLU/g (propyl laurate units) was kindly supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). The enzymes were used without further purification. The other materials used were of the highest available purity.

The number-average molecular weight (M_n), weight-average molecular weight (M_w) and molecular weight dispersion (M_w/M_n) were measured by a size exclusion chromatography (SEC) using SEC columns (for chloroform-soluble polymers: Shodex K-803L + K-8006 + K-800D, Showa Denko Co., Ltd., Tokyo, Japan, and chloroform as the eluent; for water-soluble polymers: TSK gel G5000PW + G2500PW, Tosoh Co., Ltd., Tokyo, Japan, and 0.1 mol/L phosphate buffer/0.3 mol/L sodium chloride, pH 6.8 as the eluent) with a refractive index detector. The SEC system was calibrated with polystyrene standards for Shodex columns and poly(ethylene oxide) for TSK gel columns. ^{1}H NMR spectra were recorded with a JEOL Model GSX-270 (270 MHz) spectrometer (JEOL Ltd., Tokyo, Japan). ^{13}C NMR spectra were

recorded with a JEOL model GSX-270 Fourier Transform Spectrometer operating at 67.5 MHz with complete proton decoupling. Infrared (IR) spectra were measured using a JASCO Fourier Transform Spectrometer model FT/IR-5000 (JASCO Ltd., Tokyo, Japan). The matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was measured with a Bruker Proflex mass spectrometer. The detection was in the reflection mode. 2,5-Dihydroxybenzoic acid was used as the matrix and positive ionization was used.

Conversion of BG to the polyester was determined by comparison of the ^{1}H NMR spectral integration intensities of the δ = 3.48 ppm peaks (1H) corresponding to the methyne group in oxiranes of the monomeric BG with the methylene proton of poly(BG/SA) at δ = 4.48 ppm (2H). In a similar way, conversion of GPE to the polyester was determined by comparison of the ^{1}H NMR spectral integration intensities of the δ = 3.2 - 3.5 ppm peaks (1H) corresponding to the methyne group of the monomeric GPE with the methyne proton of poly(GPE/SA) at δ = 5.15 - 5.5 ppm (1H).

Scheme 1:



Preparation of polyesters

The enzyme-catalyzed ring-opening polymerization of oxiranes and dicarboxylic anhydrides was carried out as shown in *Scheme 1*. Poly(BG/SA) was synthesized by the copolymerization of BG and SA in bulk using commercially available lipases. A typical preparation of poly(BG/SA) with an M_W of 3400 (entry 2 in Tab. 1) was carried out as follows. A mixture of porcine pancreatic lipase (PPL: 7.8 mg), BG (100 mg) and SA (56 mg) was stirred under an argon atmosphere in a capped vial placed in a thermostated oil bath at 80 °C for 5 d. After the reaction, the reaction mixture was dissolved in chloroform (6 mL), and the insoluble enzyme was removed by filtration. The chloroform was then evaporated under reduced pressure to quantitatively obtain the polymer. The monomer conversion as determined by ^{1}H NMR was 86%. The M_W relative to polystyrene and the M_W/M_n as measured by SEC were 3400 and 1.4, respectively. The molecular structure was analyzed by FT-IR, ^{1}H-NMR and ^{13}C NMR spectroscopies. The spectral data of poly(BG/SA) having an M_W of 3400 (entry 2 in Tab. 1)

are shown as being representative. IR(KBr): 2959, 1454 (CH_2), 1745, 1148 (ester C=O), 754, 700 (aromatic) cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$): δ = 2.4 - 2.8 (m; 4H, CH_2CH_2 of SA), 4.48 (d, J=4.02 Hz, 2H, $OC\underline{H}_2CH$), 5.20 (s; 2H, OCH_2Ph), 5.36 (t, J=4.02 Hz, 1H, $OCH_2C\underline{H}$), 7.34 (s, 5H, aromatic). ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ = 28.6, 62.9, 67.6, 70.6, 128.5, 128.6, 128.7, 135.1, 166.9, 171.2, 171.3.

In a similar way, poly(BG/DGA) was prepared by the copolymerization of BG and DGA in bulk using lipase. The spectral data of poly(BG/DGA) having an M_W of 1400 (entry 9 in Tab. 1) are shown as being representative. IR(KBr): 2920, 1456 (CH_2), 1755, 1194 (ester C=O), 1138 (CH_2OCH_2), 752, 700 (aromatic) cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$): δ = 4.06 - 4.32 (m; 4H, CH_2OCH_2 of DGA), 4,53 (d, J=4.02 Hz, 2H, $OC\underline{H}_2CH$), 5,18 (s; 2H, OCH_2Ph), 5,42 (t, J=4.02 Hz, 1H, $OCH_2C\underline{H}$), 7,23 (s, 5H, aromatic). ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ = 62.8, 67.8, 67.6, 70.6, 128.5, 128.6, 128.8, 134.9, 166.4, 168.9, 169,1.

Poly(GPE/SA) was also prepared by the copolymerization of GPE and SA in bulk using lipase. The spectral data of poly(GPE/SA) having an M_W of 4100 (entry 11 in Tab. 1) are shown as being representative. IR(KBr): 2938, 1495 (CH_2), 1740, 1155 (ester C=O) , 756, 693 (aromatic), 1242 (Ph-O-C) cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$): δ = 2.63 (s; 4H, CH_2CH_2 of SA), 4,06 (d, J = 4.6 Hz, 2H, $OC\underline{H}_2CH$), 4.24 - 4.46 (m, 2H, CH_2OPh), 5.15 - 5.50 (m; 1H, $OCH_2C\underline{H}$), 6.80 - 7.00 (m, 3H, aromatic), 7.18 - 7.30 (m, 2H, aromatic). ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ = 28.8, 29.0, 62.8, 65.9, 70.1, 114.7, 121.4, 129.6, 158.2, 171.6.

Debenzylation was carried out basically according to the method of Anantharamaiah et al. [38]. Poly(BG/SA) (500mg) was dissolved in a 20 mL tetrahydrofuran (THF), and to this 5% Pd on carbon (500 mg) and cyclohexene (10 mL) were added and refluxed for 3 h. After filtration of Pd/C catalyst, organic solvent was removed by vacuum evaporation to yield debenzylated polyester. The polymer was dissolved in THF, (5 mL), then added 5 mL water and neutralized by the addition of 10% sodium bicarbonate to pH 7.5. The neutralized solution was dialyzed against distilled water for 2 h and then lyophilized to obtain poly(SG/SA) (425 mg) in 85% yield. IR(KBr): 2959, 1412 (CH_2), 1732, 1163 (ester C=O), 1622 (COONa) cm^{-1}. ^{1}H-NMR (270 MHz, D_2O): δ = 2.7 (t; J=4.82 Hz, 4H, CH_2CH_2 of SA), 4.47 (d, J=3.80 Hz, 2H, $OC\underline{H}_2CH$), 5,12 (t, J=3.80 Hz, 1H, $OCH_2C\underline{H}$). ^{13}C-NMR (67.5 MHz, D_2O): δ = 29.5, 65.3, 74.6, 174.3, 174.8, 175.2. In a similar way poly(SG/DGA) was prepared. IR(KBr): 2959, 1412 (CH2), 1753, 1213 (ester C=O), 1630 (COONa), 1140 (CH_2OCH_2) cm^{-1}. ^{1}H-NMR (270 MHz, D_2O): δ = 4.11-4.53 (m, 4H, CH_2OCH_2 of DGA), 4.67 (d, J=3.77 Hz, 2H, $OC\underline{H}_2CH$), 5.21 (t, J=3.77 Hz, 1H, $OCH_2C\underline{H}$). ^{13}C-NMR (67.5 MHz, D_2O): δ = 65.3, 68.3, 68.7, 74.6, 171.9, 172.1, 173.7.

In order to compare the physicochemical properties of poly(BG/SA) having molecular weights greater than 8000, polymerization of BG with SA was carried out using aluminum isopropoxide instead of the lipase catalyst. The 10 wt% aluminum isopropoxide (15% toluene solution) was used as a catalyst for the polymerization. A typical polymerization was as follows. An equimolar mixture of BG and SA containing 0.1 mol-% aluminium isopropoxide was polymerized under an argon atmosphere at 100 °C for 5 d. The polymer was purified by the

reprecipitation using chloroform as a good solvent and diethyl ether as a poor solvent to yield poly(BG/SA). In a similar way, poly(BG/DGA) was prepared. The obtained poly(BG/SA) and poly(BG/DGA) showed identical spectral properties with that obtained when using the lipase catalyst. They were debenzylated to form the corresponding sodium salt as already described and their physicochemical properties and biodegradability were then evaluated.

Hydrolytic Degradation

A non-enzymatic degradation test was carried out by dissolving the polymer in buffer solutions with pH values of 4, 7 and 9. The polymers at a concentration of 400 mg/L were incubated at 30 °C in an incubator. Solutions of 0.1 M acetate buffer at pH 4.0, 0.1 M phosphate buffer at pH 7.4, and 0.1 M tris-HCl buffer at pH 9.0 were used for the hydrolytic degradation test. The degradation of the polymer was analyzed by SEC before and after the incubation.

Calcium Ion Sequestration Capacity

A calcium ion electrode (Model 93-20, Orion Research, Inc., Boston, MA) and an ion meter (IM-20E, TOA Electronic, Ltd., Tokyo, Japan) were used to measure the equilibrium calcium ion concentrations. Ten milligrams of the polymer was dissolved in 50 mL of 1.00 x 10^{-3} M calcium hardness solution containing a 0.08 M KCl (ion strength, $\mu = 0.08$). The pH of the solution was adjusted to 9.0 at 30 °C. The electrode was immersed in the solution, which was then stirred. After 10 minutes, equilibrium free calcium ion concentrations were measured, and the calcium sequestration capacity was expressed as grams of calcium ion sequestered by 100 g of polymer.

Biodegradation Test

Biochemical oxygen demand (BOD) was determined with a BOD tester (Model 200 and 100 F; TAITEC Corp., Koshigaya-Shi, Japan) by the oxygen consumption method basically according to the OECD Guidelines for Testing of Chemicals (301C, Modified MITI Test) at 25 °C, using an activated sludge freshly obtained from a municipal sewage treatment plant in Yokohama city. The concentration of the polymer in the incubation media was 25 mg/L.

Results and discussion

Enzymatic polymerization of oxiranes and dicarboxylic anhydrides

Tab. 1 shows the typical copolymerization of BG and dicarboxylic anhydride by the enzymatic catalyst and also by the chemical catalyst as a comparison. It was found that BG was copolymerized with SA and DGA in bulk in the presence of lipase to yield the corresponding polyesters bearing carboxyl groups. The polymerization occurred with all lipases tested. However, a significant difference between the enzymes was observed with respect to the M_w and the monomer conversion. Among the lipases tested, PPL showed the highest activities for the copolymerization. GPE was also used as the oxirane monomer in order to analyze the lipase-catalyzed copolymerization of oxiranes and dicarboxylic anhydride, and the polymerization results are summarized in Tab. 1.

Tab. 1: Conditions and results of the ring-opening copolymerization of oxiranes and acid anhydride[a)]

Entry[b)]	Oxirane	Acid anhydride	Catalyst[c)]	Conc. of catalyst in wt.-% [d)]	Temp. in °C	Time in day	Conv. in %	$\overline{M}_w$[e)]	$\overline{M}_w/\overline{M}_n$
1	BG	SA	---	0	80	5	0	--- [f)]	---
2	BG	SA	PPL	5	80	5	86	3400	1.4
3	BG	SA	PPL	15	80	5	95	2300	1.5
4	BG	SA	CC	5	80	5	78	2200	1.5
5	BG	SA	PS	5	80	5	17	1050	1.1
6	BG	SA	Novo	5	80	5	3	---	1.1
7	BG	SA	PPL	5	60	5	15	2300	1.6
8	BG	DGA	PPL	2	80	5	7	1000	1.2
9	BG	DGA	CC	2	80	5	10	1400	1.4
10	GPE	SA	---	0	80	5	0	---	---
11	GPE	SA	PPL	5	80	5	99	4100	1.9
12	GPE	SA	CC	5	80	5	100	3500	1.7
13	GPE	SA	PS	5	80	5	33	2500	1.3
14	GPE	SA	Novo	5	80	5	17	1900	1.2
15	GPE	SA	PPL	5	60	5	39	2700	1.7
16	BG	SA	AIP	0.1	100	2	88	9100	1.6

[a)] Bulk polymerization of oxirane and acid anhydride (succinic anhydride: SA; diglycolic anhydride: DGA) with a molar ratio of 1:1. GPE: glycidyl phenyl ether, BG: benzyl glycidate.
[b)] Entries 1 and 10: blank tests.
[c)] PPL: Porcine pancreatic lipase, CC: *Candida cylindracea* lipase, PS: Lipase PS, Novo: Novozym 435. AIP: aluminum isopropoxide (15% toluene solution).
[d)] The lipase concentration based on the two monomers.
[e)] Determined by SEC analysis, calibrated with polystyrene standards.
[f)] Polymer fraction by SEC was not detected.

The polymerization activity of PPL remained unchanged after 3-h stand at 80 °C; however, the activity disappeared by boiling in aqueous sodium dodecylsulfate (SDS) solution for 1 h. It was also confirmed that oxiranes and SA remained unchanged without lipase at 60 and 80 °C after a 5-d incubation, indicating that the enzyme actually promoted the copolymerization. Purified porcine pancreatic lipase having 30000 unit/mg protein was used in order to compare the polymerization results using the crude PPL powder having 190 unit/mg protein. It was confirmed that a similar copolymerization of BG and SA occurred using both purified porcine pancreatic lipase and crude PPL powder on the same unit basis. This indicates that the PPL enzyme actually catalyzed the copolymerization of BG and SA and no significant effects by the impurities in the crude PPL powder were observed. Therefore, in this report, crude PPL powder was used for all subsequent studies.

It was found that the M_W of the resulting polymer was dependent on the reaction temperature, enzyme concentration and reaction time. The M_W increased with increasing polymerization temperature from 60 to 80 °C, but decreased slightly at 100 °C. Fig. 1 shows the relationship between the M_W and monomer conversion with the enzyme concentration of PPL and CC of the bulk copolymerization of oxiranes and SA with a molar ratio of 1:1 at 80 °C for 5 d. The maximum M_W of poly(BG/SA) was obtained at a lipase concentration of around 3-5% both for PPL and CC at 80 °C. The M_W of the polyester decreased with increasing lipase concentration from 5 to 30%. On the other hand, the monomer conversion was quickly increased at the enzyme concentration of around 5% and then gradually increased with increasing enzyme concentration. Similar results were obtained for the polymerization of GPE and SA using PPL as shown in Fig. 2. That is, the maximum M_W of poly(GPE/SA) was obtained at a lipase concentration of 3% PPL at 80 °C for a 5-d polymerization. The M_W of the polymer decreased with increasing lipase concentration of greater than 3% PPL.

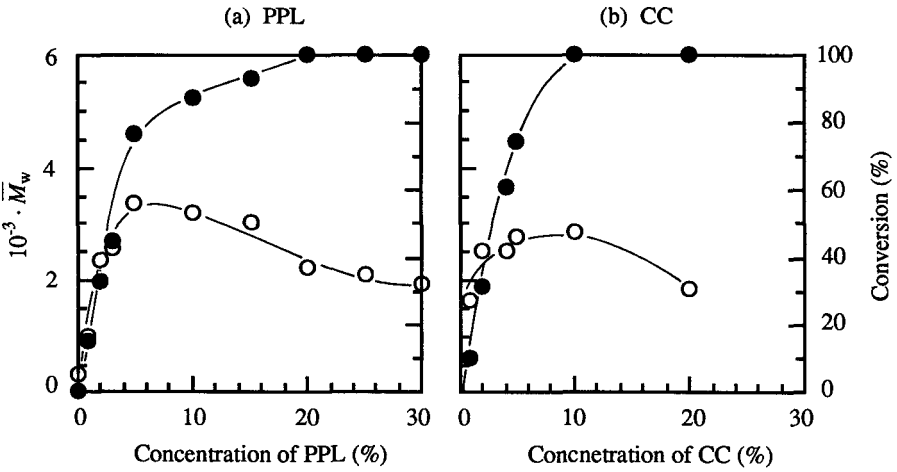


Fig. 1: Relationship between molecular weight $\bar{M}_w$ and monomer conversion of BG and SA with a molar ratio of 1:1 in bulk at 80 °C for 5 d as a function of lipase concentration
○ : $\bar{M}_w$, ● : monomer conversion (%)

Fig. 3 shows the time course of the polymerization of BG and SA using 5% PPL at 80 °C. It was found that the M_W of the polymer gradually increased until 5-6 d and then slowly decreased. This is probably ascribed to the reverse or degradation reaction during the prolonged polymerization. Similar tendencies were observed for the polymerization of GPE and SA using 5% PPL at 80 °C. Both the M_W and monomer conversion were quickly increased within the first 1 d and then slowly increased.

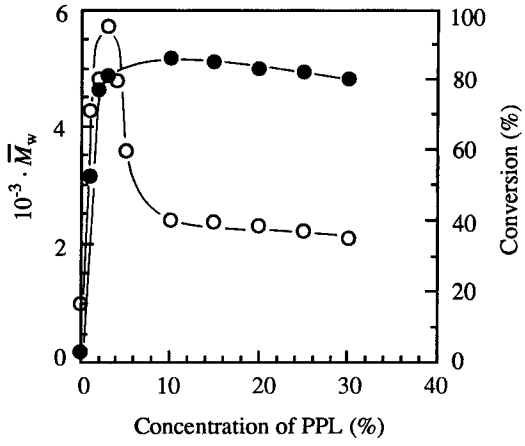


Fig. 2: Lipase-catalyzed polymerization of GPE and SA by PPL for 5 d at 80 °C in bulk ○ : $\overline{M}_w$, ● : monomer conversion (%)

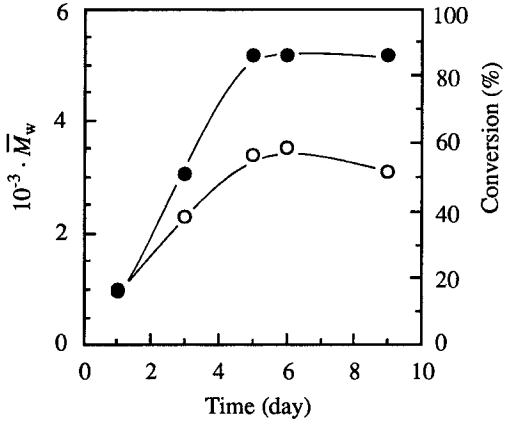


Fig. 3: Lipase-catalyzed polymerization of BG and SA by 5% PPL at 80 °C in bulk ○ : $\overline{M}_w$, ● : monomer conversion (%)

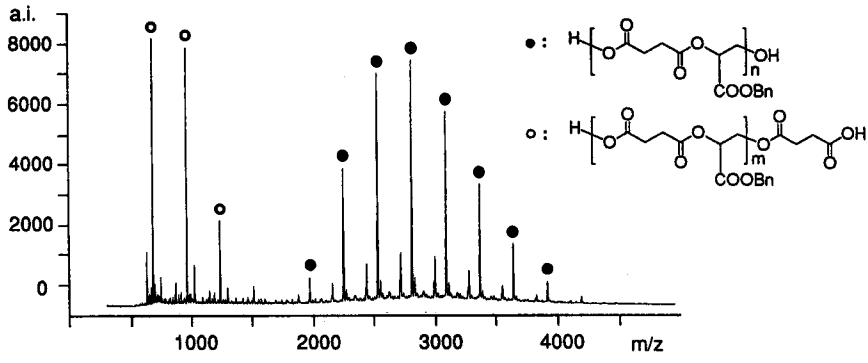


Fig.4: MALDI-TOF mass spectrum of poly(BG/SA), recorded in reflector mode using DHBA as matrix. Bulk polymerization of BG and SA using 5% PPL at 80°C for 40 h

Fig. 4 shows the typical MALDI-TOF MS spectrums for poly(BG/SA) of 40 h polymrization using 5% PPL at 80 °C. The MALDI-TOF MS showed that the repeating units have a mass of 278 m/z, which confirmed the assigned structure. The ionization process of a neutral macromolecule in MALDI-TOF MS proceeds through the capture of a proton or a metal ion, usually sodium, which forms a charged adduct with the molecular species [39]. The spectrum could be grouped into two types of spectrum according to the end groups of the polymer chain as shown in Fig. 4. It was found that both SEC and MALDI-TOF MS of the polymerization mixture of BG and SA showed two peaks for the polymer fractions of poly(BG/SA). The lower molecular weight peak of SEC having a peak-top molecular weight of around 1000 remained below 2000 during the 9-d polymerization. On the other hand, the high molecular weight peak of SEC gradually shifted towards a higher molecular weight with polymerization time. Fig. 5 shows the time course of the peak-top molecular weight increase of the lower molecular weight fractions and the higher molecular weight fractions obtained from the MALDI-TOF MS.

According to the mechanism proposed for the lipase-catalyzed polymerization of lactones, the polymerization was initiated by the reaction of the enzyme with the lactone to form an acyl-enzyme complex, or enzyme-activated monomer. The enzyme-activated monomer then reacts either with a nucleophile to accomplish the initiation or with the hydroxyl groups of a growing polymer chain to continue the propagation [40-42]. These mechanisms will be basically applicable to the copolymerization of BG and SA as shown in Fig. 6. That is, the polymerization was initiated by the reaction of the enzyme with succinic anhydride to form an enzyme-activated succinate (EAS) which was followed by the reaction with BG to form an enzyme-activated monomeric ester unit (EAM). This EAM may be an active species for a growing polymer chain. The produced polymer had a carboxyl and hydroxyl group at the terminal of the polymer chain. However, EAS may also react with a growing oligomer to

produce an oligomeric species having SA-derived carboxyl groups at both ends. The reactivity of the oligomers having carboxyl groups at the both terminals might be lower when compared to the oligomers having the hydroxyl group at a terminal. Oligomers having a hydroxyl group at the chain end might be more quickly successively reacted with EAM to increase its molecular weight.

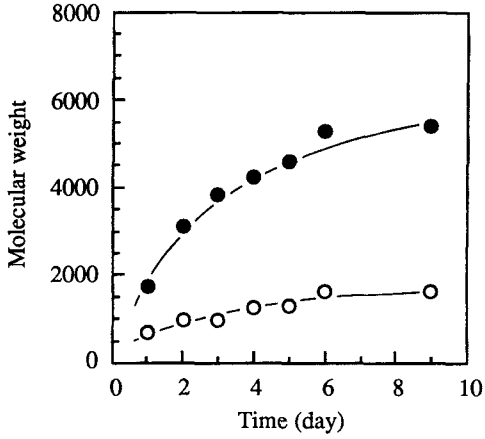


Fig. 5: Molecular weights (peak top) of high-molecular weight fraction and low-molecular weight fraction produced by the polymerization of BG and SA by 5% PPL at 80 °C in bulk. ●: high-molecular weight, ○: low-molecular weight

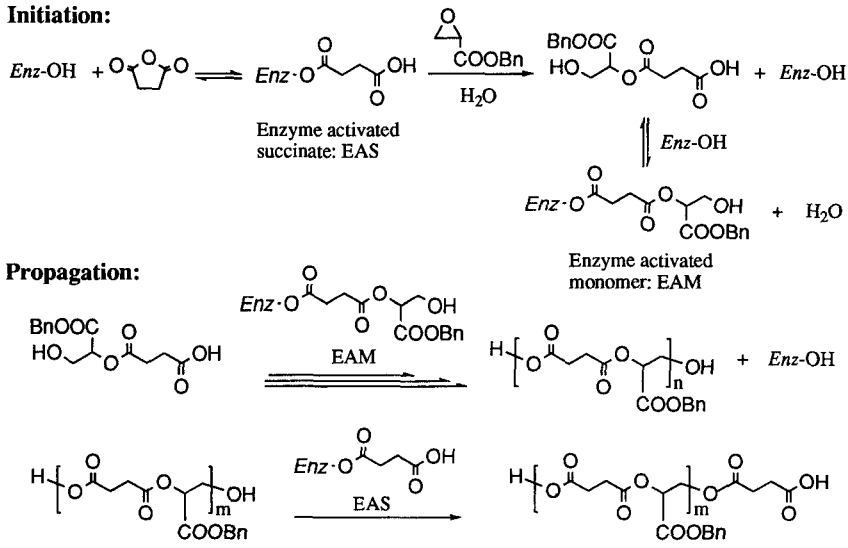


Fig. 6: Proposed basic mechanism of enzymatic polymerization of BG and SA

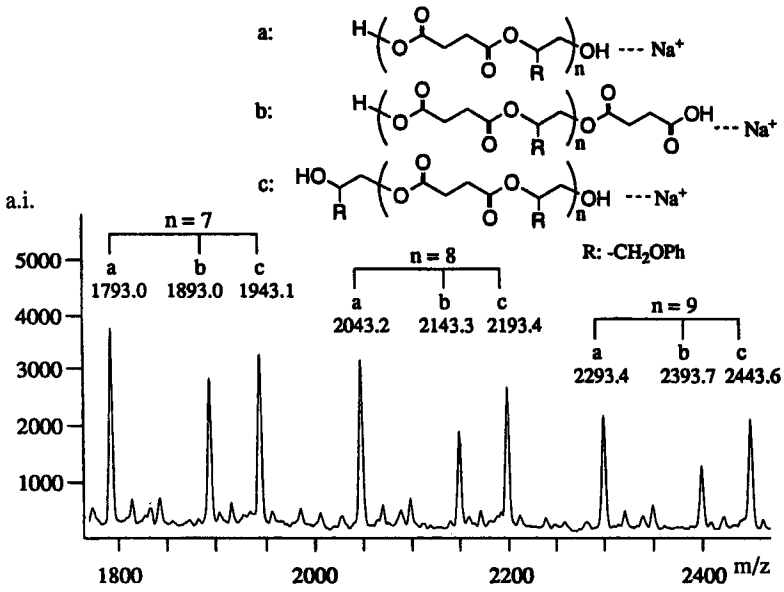


Fig. 7: MALDI-TOF mass spectrum of poly(GPE/SA), recorded in reflector mode using DHBA as matrix. Bulk polymerization of GPE and SA using 5% PPL at 80°C for 2 d

In case of the polymerization of GPE and SA, the polymerization occurred without formation of lower oligomeric fractions having carboxyl groups at the both terminals of the chain. Fig. 7 shows the typical MALDI-TOF MS spectrum for poly(GPE/SA) after 2-d polymerization at 80 °C. The MALDI-TOF MS showed that the repeating units have a mass of 250 m/z, which confirmed the assigned structure. The spectrum could be grouped into three types of spectrum each having 250, 150 and 100 m/z of peak separations according to the end groups of the polymer as shown in Fig. 7. The different terminal groups of the resulting polyester might be ascribed to the ester-exchange reactions or degradation reactions that occurred in parallel to the polymerization process.

In order to get higher molecular weight of the polyester, the addition of the monomer during the polymerization may be an effective way. By the addition of the two monomers into the solidified polymer mixture, further polymerization will be occurred in the dissolved polymer mixture. Therefore, the same quantity of the two monomers was added to the solidified polymer mixture after 1-d to 5-d polymerization in order to facilitate the polymerization. The results are shown in Tab. 2. It was confirmed that the M_W of the polyester increased due to the addition of the two monomers after 1-d to 5-d polymerization. The Maximum M_W of 10600 was attained by the addition of the two monomers during the polymerization. That is, GPE and SA with a molar ratio of 1:1 was polymerized by 5% PPL at 80 °C for 5 d. The same quantity of the two monomers of GPE and SA with a molar ratio of 1:1 was then added to dissolve the solidified

polymer mixture, and stirred for another 3 d at 80 °C. The M_W of poly(GPE/SA) as determined by SEC was 10600 and the monomer conversion was 99% after 8-d polymerization.

Tab. 2: Two-step copolymerization of glycidyl phenyl ether and succinic anhydride[a)]

Entry[b)]	Polymerization time in day		Conv. in %	$\overline{M}_w$[c)]	$\overline{M}_w/\overline{M}_n$
	Primary polymerization	Polymerization after addition of the two monomers			
1	5	---[b)]	99	4100[c)]	1.9
2	2	3	92	7200	2.1
3	4	2	99	8800	2.5
4	5	3	99	10600	2.4
5	8	---	99	7000	3.0

[a)] Bulk polymerization of glycidyl phenyl ether (GPE) and succinic anhydride (SA) with a molar ratio of 1:1 using 5% porcine pancreatic lipase (primary polymerization basis) at 80 °C. The lipase concentration based on the two monomers.
[b)] Entries 1 and 5: without addition of two monomers during polymerization.
[c)] Determined by SEC analysis, calibrated with polystyrene standards.

Physicochemical properties and biodegradability as water-soluble polyesters baring carboxylate groups

Poly(BG/SA) and poly(BG/DGA) were readily debenzylated by hydrogenolysis using cyclohexene/THF in the presence of Pd/C to yield the water-soluble poly(sodium carboxylate)s. Their physicochemical properties, such as hydrolytic degradability and calcium sequestration capacity, and biodegradability were evaluated as water-soluble carboxylate-type polyesters.

The non-enzymatic hydrolytic degradability of the polymers was evaluated in sterile aqueous solutions of different pH values. Fig. 8 shows the representative set of SEC curves demonstrating the use of the molecular weight reduction. It was found that a significant molecular weight reduction was observed for poly(SG/DGA) by the hydrolytic cleavage at pH 4.0. The rate of molecular weight reduction increased with increasing pH of the aqueous solution. At pH 9.0, the polymer fraction of poly(SG/DGA) completely disappeared within 1 d. On the other hand, poly(SG/SA) was practically stable in the pH range of 4 and 7.4. However, at pH 9 in a slightly alkaline aqueous solution, the polymer fraction was gradually cleaved by hydrolysis to produce lower molecular weight fractions.

Calcium ion sequestration capacity is one of the attractive properties for polycarboxylate-type, water-soluble polymers. Fig. 9 shows the calcium ion sequestration capacity of poly(SG/SA) as a function of molecular weight. It was found that the calcium ion sequestration capacity was relatively lower than that of poly(sodium acrylate) when compared on the same molecular weight basis. This is probably ascribed to the ester bonds which inhibited free rotation for effective calcium ion sequestration.

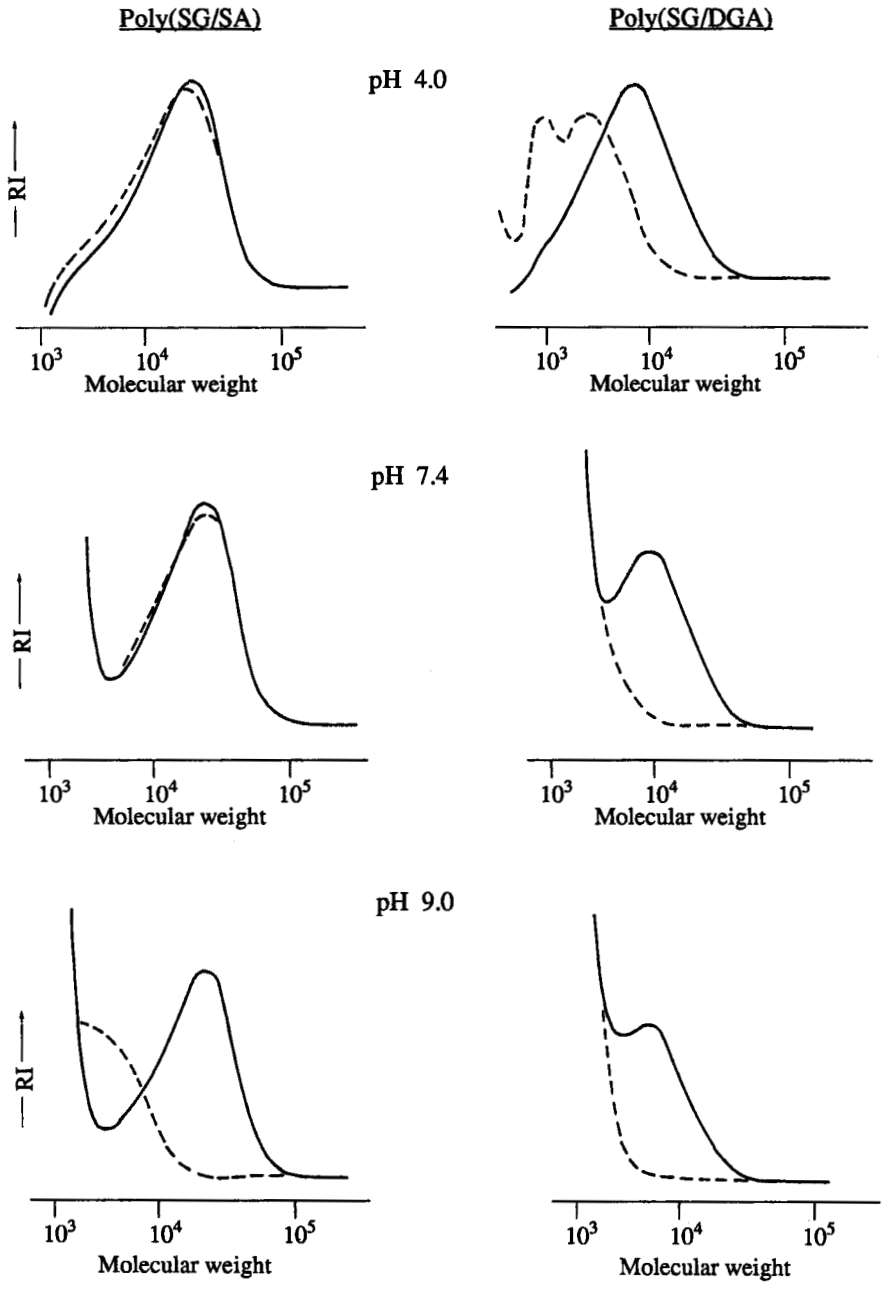


Fig. 8: SEC profile changes of poly(SG/SA) and poly(SG/DGA) before and after the hydrolysis — : initial, --- : 4-d incubation

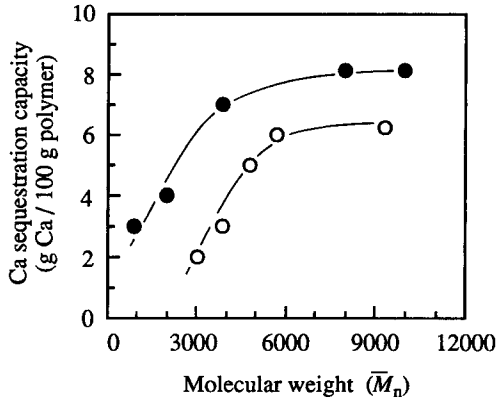


Fig. 9: Ca sequestration capacity as a function of molecular weight
o : poly(SG/SA), ● : poly(sodium acrylate)

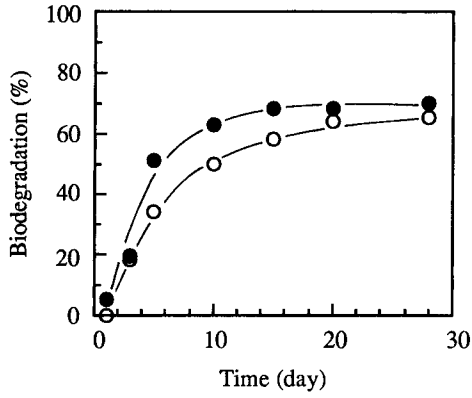


Fig. 10: Biodegradation (BOD/ThOD x 100) using activated sludge
o : poly(SG/SA), ● : poly(SG/DGA)

Ready and complete biodegradation after use is one of the indispensable factors for water-soluble polymers in the next generation, because water-soluble polymers are generally difficult to recover. A convenient way of predicting the aerobic biodegradability of the polymers is to measure the BOD values. Biodegradability of the polymers was evaluated by measuring BOD and TOC values using activated sludge. Fig. 10 shows the time course of biodegradation of the polymers by the activated sludge at 25°C. The BOD values were measured with a BOD tester using an activated sludge freshly obtained from a municipal sewage treatment plant and a test substrate concentration of 25 mg/L. It was confirmed that all polymers tested in this study were biodegraded to over 60% BOD-biodegradation in 28 d, which is the criterion for ready

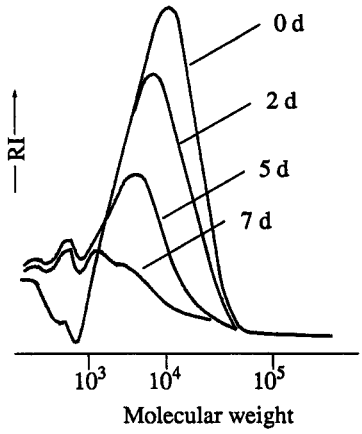


Fig. 11: SEC profile changes of poly(SG/SA) before and after the biodegradation using activated sludge

biodegradation, suggesting that these compounds are biodegradable in the environment. The difference in the biodegradability between the two polymer series was not clearly obseved. Also, from the TOC biodegradability, almost all organic carbon was removed from the biodegradation media, and no biodegradation intermediate was accumulated by the biodegradation. Measuring the molecular weight and molecular weight distribution of the polymer by SEC before and after the biodegradation test is a useful tool to determine the main-chain scission of the polymers as well as to establish the overall degradation of the polymers. For the latter, the peak area on SEC roughly corresponds to the polymer concentration in the biodegradation medium. Fig. 11 shows the SEC profiles of the cell-free culture filtrate before and after the biodegradation test in the BOD tester. The SEC peaks of the polymers completely disappeared.

Conclusions

It was found that oxiranes and dicarboxylic anhydrides were readily polymerized in the presence of lipase at a temperature between 60 and 80 °C to yield the corresponding polyesters. Among the lipases tested, PPL showed the best results with respect to the monomer conversion and the molecular weight of the resulting polymer. The most preferable concentration of lipase PPL and the reaction temperature were about 3-5% and 80 °C, respectively. It was confirmed that poly(SG/SA) polymers were readily biodegraded by the activated sludge, and were more stable against hydrolysis in an aqueous solution of pH 4 to 7 than the poly(sodium malate).

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