Novel Enzyme-Catalyzed Ring-Opening Polymerization of Glycidol

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Glycidol polymerized by the microbial resting cells and commercially available hydrolase enzyme having epoxide hydrolase activity without added water produced the water-soluble poly(glycidol). As a typical example, a mixture of glycidol and 5% lipase AK *(Pseudomonas fluorescens)* was stirred at 30 °C for 3 d to produce the poly(glycidol) with a weight-average molecular weight of 900.

Relatively low-molecular-weight poly(glycidol) (polyglycerol) has attracted attention as water-soluble hydrophilic nonionic oligomers which may have potential application in the field of surfactants and cosmetics.[1] Epoxide hydrolases catalyze the hydrolysis of epoxides to form the corresponding diols without a cofactor. These enzymes have been shown to widely occur in mammals, plants, insects, fungi, and bacteria. It is reported that epoxide hydrolase was isolated from *Rhodococcus* sp. NCIMB 11216 and its hydrolysis activity was evaluated using a series of structurally different epoxides.[2,3] Hechtberger et al. reported the asymmetric hydrolysis of epoxides using an immobilized crude enzyme preparation derived from *Rhodococcus* sp. to produce the corresponding 1,2-diol.[4] Increasing attention is given to the potential use of epoxide hydrolases in the biocatalytic production of enantiopure epoxides from racemates.[5] It was shown that liver microsomes may be used in the stereoselective ring opening of epoxides with amines.[6] More recently, it has been reported that some commercially available lipases, such as porcine pancreatic lipase which is applicable on a preparative scale, catalyzed the enantioselective ring opening of epoxides with 2-propylamine.[7] However, enzymatic polymerization of an epoxide has not yet been reported. In this report, the enzyme-catalyzed ring-opening polymerization of 2,3-epoxypropanol (glycidol) was carried out using the microbial resting cells and commercially available hydrolase as the epoxide hydrolase source.

First, various kinds of hydrolase enzymes were screened with respect to the epoxide hydrolase activity using 30% aqueous glycidol solution with 10% enzyme (wt% to glycidol) at 40 °C for 1 day. As a reference, resting cells of *Rhodococcus* sp. NCIMB 11216 as the epoxide hydrolase source were prepared according to the literature.[8,9] The results are summarized in Table 1. It was found that glycidol was hydrolyzed by the commercially available hydrolases and the microbial epoxide hydrolase obtained from *Rhodococcus* sp. Without the enzyme, practically no hydrolysis occurred. The rate of hydrolysis was dependent on the enzyme origin. Among the enzymes tested, lipase AK and the resting cells of *Rhodococcus* sp. showed good hydrolysis of glycidol in aqueous solutions. It was found that under limited water concentrations, without added water, glycidol polymerized by the enzyme having epoxide hydrolase activity produced the water-soluble poly(glycidol). In this case,

Table 1. Enzyme-catalyzed hydrolysis and polymerization of glycidol at 40 °C

Enzyme origin[a,14]	Hydrolysis[b]	Polymerization[c]		
	Conv./%	Conv./%	M_w	M_w/M_n
Pseudomonas fluorescens (1)	40	99	900	2.2
Pseudomonas sp. (2)	30	99	470	1.5
Porcine pancreatic lipase	22	63	<300	1.4
Candida antarctica (3)	9	15	<300	1.2
Candida rugosa (4)	18	8	<300	1.3
Rhodococcus sp. (5)[d]	36	99	440	1.5
Blank	5	5	--	--

(1) lipase AK (2) lipase PS (3) lipase CA (4) lipase CR [a](5) resting cells of *Rhodococcus* sp. NCIMB 11216. [b]Hydrolysis of glycidol: 10 eq. H_2O and 10% enzyme at 40 °C for 1 d. [c]Bulk polymerization of glycidol: 10% enzyme at 40 °C for 3 d. [d]Hydrolysis and polymerization: using 10% resting cell at 30 °C.

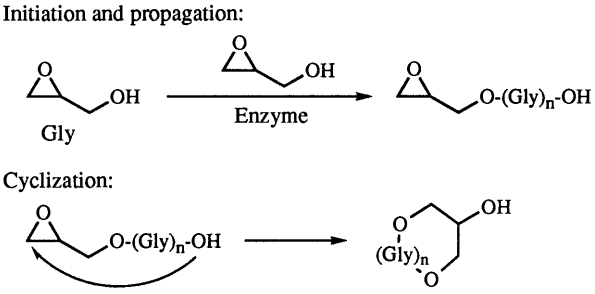

Figure 1. Polymerization of glycidol (Gly).

the hydroxy group of glycidol may act as a nucleophile instead of water to initiate the ring-opening polymerization of glycidol as shown in Figure 1. On the other hand, by the addition of organic solvents, such as DMF and acetone, polymerization of glycidol significantly decreased by the deactivation of the enzyme. The monomer conversions were 2 and 9% in DMF and acetone, respectively.[10] Furthermore, without an enzyme, no clear oligomerization occurred at 30 and 40 °C, indicating that the enzyme actually promoted the polymerization of glycidol. As a typical example, poly(glycidol) with a weight-average molecular weight of 900 was prepared in bulk as follows. A mixture of glycidol and 5% lipase AK *(Pseudomonas fluorescens)* as the epoxide hydrolase was stirred under an argon atmosphere in a capped vial placed in an oil bath at 30 °C for 3 days. After the reaction, the reaction mixture was dissolved in methanol, and the insoluble enzyme was removed by filtration. The solvent was then evaporated under reduced pressure to quantitatively obtain the polymer mixture. The polymer structure of poly(glycidol) was analyzed using ^{1}H NMR, ^{13}C NMR, DEPT-NMR, MALDI-TOF MS and SEC.[11] It was confirmed

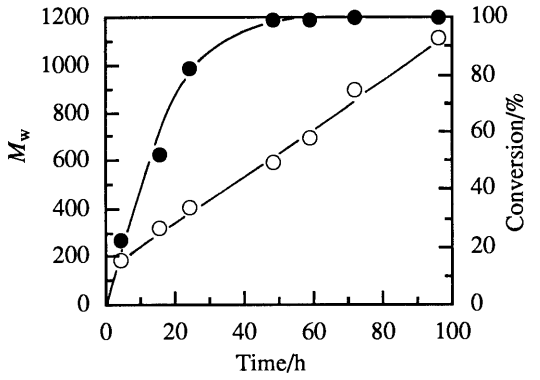

Figure 2. Time course of polymerization of glycidol using 5% lipase AK at 30 °C. ○: M_w, ●: Monomer conversion.

that the resulting poly(glycidol) had branching structures and either an epoxide group at the terminal or macrocyclic structure.

Figure 2 shows the time-course of the polymerization of glycidol using 5% lipase AK at 30 °C in bulk. The monomer conversion quickly increased to 100% after 48 h by lipase AK. The molecular weight of the resulting polymer gradually increased with time. After 100% monomer conversion, the molecular weight of the resulting polymer still increased. This may be ascribed to the intermolecular reaction between hydroxy group and terminal epoxide group of the polymer chain.

Figure 3 shows the MALDI-TOF MS spectrum of poly(glycidol) obtained by the bulk polymerization of glycidol using 5% lipase AK at 40 °C for 3 days. It was confirmed that the repeating units have a mass of *m/z* 74.1 , which confirms the polymer structure shown in Figure 1.[12] Also, each peak of the MALDI-TOF MS was corresponded to the poly(glycidol) having either epoxide terminal or macrocyclic structure with one Na^+ ion. In order to further confirm the existence of the terminal epoxide group in poly(glycidol), the reactive epoxide group of poly(glycidol) was subjected to hydrolysis using aqueous NaOH at 80 °C for 18 h. It was found that a series of new peaks were appeared on MALDI-TOF MS after hydrolysis. The mass differences of *m/z* 18 were observed between original and newly appeared peaks. These results indicated that the reactive terminal epoxide group was hydrolyzed to form the corresponding diols. On the other hand, the remaining peaks after hydrolysis corresponded to the macrocyclic poly(glycidol).

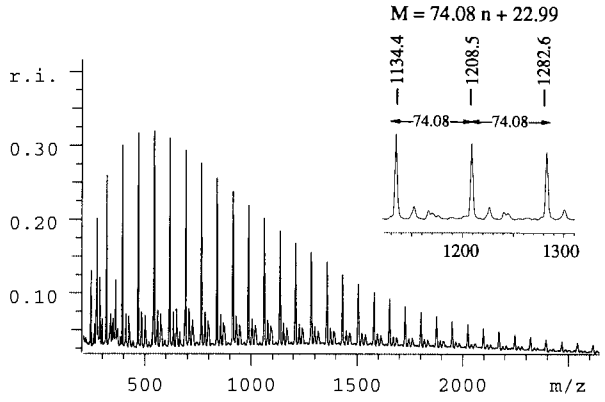

Figure 3. MALDI-TOF MS spectrum of poly(glycidol) obtained by the bulk polymerization of glycidol using 5% lipase AK at 40 °C for 3 days.

The existence of the terminal epoxide group was also confirmed by ^{1}H NMR (300 MHz: CD_3OD) such that 2.61 ppm and 2.77 ppm are ascribed to the methylene protones of the epoxide ring. After hydrolysis of poly(glycidol), 2.61 and 2.77 ppm peaks ascribed to the epoxide groups disappeared. The content of the terminal epoxide was decreased with polymerization time and macrocyclic poly(glycidol) was the major component after 72 h. The assignment of signals by the ^{13}C NMR spectra of the poly(glycidol) obtained by the cationic polymerization of glycidol was based on the literature.[13] The DEPT NMR spectra of poly(glycidol) obtained by the bulk polymerization of glycidol using 5% lipase AK at 40 °C for 3 days indicated that poly(glycidol) had 1,3- and 1,4-linkages and also dendric moieties. Details of the mechanism for the enzyme-catalyzed polymerization of oxiranes are now under study.

Further work should reveal the influence of the polymerization conditions as well as enzyme origin on the ratio of *sec*-OH/*pr*-OH. Studies of the enzymatic polymerization of glycidol may lead to a better understanding of the conditions required to prepare higher molecular weight products with the desired macromolecular architecture.

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References and Notes

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10 Polymerization was carried out using 5% lipase AK (wt% to glycidol) and 20% glycidol in organic solvent at 30 °C for 72 h.
11 The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were measured by a size-exclusion chromatography (SEC) using SEC columns (TSKgel α-3000, TOHSO Co., Ltd., Tokyo) with a refractive index detector. *N,N*-Dimethylformamide was used as the eluent. The SEC system was calibrated with poly(ethylene glycol) standards.
12 MALDI-TOF mass spectrum was measured on a Brucker Protein TOF in reflection mode using 2,5-dihydroxybenzoic acid as the matrix.
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14 Lipase PS and lipase AK "Amano"20 were kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Porcine pancreatic lipase (41 U/mg protein, according to the supplier) and lipase CR (500 U/mg, according to the supplier) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Immobilized lipase from *Candida antarctica* (CA; Novozym 435) having 7000 PLU/g (propyl laurate units) was kindly supplied by Novo Nordisk Bioindustry Ltd. (Chiba, Japan). The enzymes were used without further purification.