Preparation of Poly(β-malic acid) by Enzymatic Ring-Opening Polymerization of Benzyl β-Malolactonate

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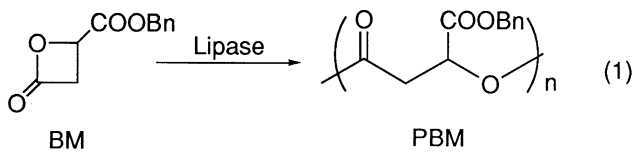
Poly(β-malic acid) was prepared by lipase-catalyzed ring-opening polymerization of benzyl β-malolactonate with subsequent debenzylation. Benzyl β-malolactonate was readily polymerized by porcine pancreatic lipase or Novozyme 435 lipase at 60 °C to yield poly(benzyl β-malate) having a molecular weight of greater than 7000.

High-molecular weight polycarboxylates have been shown to have excellent properties both in the industrial and biomedical fields. However, they are generally highly resistant to biodegradation which is an important criterion in large scale applications. Only a few polymers which contain ester or amide linkages in the backbone, such as poly(malic acid) and poly(aspartic acid), are biodegradable.[1,2] Poly(malic acid) is a biodegradable and bioadsorbable water-soluble polyester having modifiable pendant carboxylic groups. Recently, this polymer was attracted attention as a polymer carrier which is able to covalently attach drug units and targeting agents in the pharmaceutical fields,[3] and can also be used as a biodegradable raw material for the chemical industries such as detergent builders and chelating agents.[4] The chemical method for the preparation of poly(malic acid), first reported by Vert and Lenz, showed that the ring-opening polymerization of benzyl β-malolactonate is involved.[5,6] The ring-opening polymerization of the lactone requires extremely pure monomers and anhydrous conditions as well as a long reaction time. Furthermore, the polymerization catalyst may be present in the resultant polymer, and additional purification procedures will be needed for medical applications. To avoid these difficult restrictions for ring-opening polymerization of benzyl β-malolactonate by the chemical methods, enzyme-catalyzed polymerization may be one of the feasible methods to obtain poly(malic acid).

Enzymatic ring-opening polymerization of six and seven-membered lactones was first conducted using lipase as a catalyst.[7] Also, the enzyme-catalyzed ε-caprolactone ring-opening polymerization was published.[8] However, ring-opening polymerization and copolymerization using lipase were restricted to lactones greater than 6-membered lactones. The enzymatic polymerization of four-membered lactones as well as β-malolactonate has not been reported.

In this report, preparation of poly(β-D,L-malic acid) by the lipase-catalyzed ring-opening polymerization of four-membered benzyl β-D,L-malolactonate (BM) was studied.

Lipase-catalyzed ring-opening polymerization of BM was carried out as shown in scheme 1.



(1)

BM PBM

Table 1. Typical lipase-catalyzed ring-opening polymerization of benzyl β-D,L-malolactonate (BM)[a]

Entry	Lipase[b]	%[c]	Solvent	Temp /°C	Time /d	Yield /%	$\bar{M}w$	$\bar{M}w/\bar{M}n$
1	PP	10	---	60	3	60	5000	1.4
2	PP	2.5	---	60	3	83	7000	2.0
3	Novo	10	---	60	3	17	7200	1.5
4	Novo	5	---	60	3	26	6500	1.9
5	Novo	2.5	---	60	3	26	6600	1.5
6	PP	10	Isooctane	60	3	90	7200	1.8
7	PP	5	Heptane	60	3	61	7200	1.9
8	Novo	10	Isooctane	60	3	17	4500	1.4
9	Novo	5	Isooctane	60	3	22	4200	1.4
10	Novo	5	Heptane	60	3	9	7200	1.6
11	Betaine	0.12	---	40	19	30	4200	1.1
12	Betaine	0.03	---	40	29	42	4400	1.1
13	---	---	---	60	3	0	---	---

[a] Entry 2: $[\alpha]_D^{29}$ + 0.33 (c 0.92, $CHCl_3$), Entry 9: $[\alpha]_D^{30}$ + 1.80 (c 1.02, $CHCl_3$), Entry 13: blank test.
[b] PP: porcine pancreatic lipase, Novo: Novozyme 435.
[c] Weight % of lipase to BM.

A mixture of lipase[9] and BM[10] was stirred with or without heptane or isooctane as the solvent in a sealed tube placed in a thermostated oil bath. After the reaction, the reaction mixture was dissolved in chloroform, and the insoluble enzyme was removed by filtration. The organic solvent was then evaporated under reduced pressure to obtain the polymer. The polymer was purified by reprecipitation (chloroform as a good solvent; methanol as a poor solvent) to yield poly(benzyl β-D,L-malate) (PBM). The molecular weight and the conversion were analyzed by GPC.[11] The chemical polymerization of BM was carried out in a similar way except that betaine was used in place of the enzyme as the polymerization catalyst.

It was found that BM was readily polymerized by the lipase to yield PBM with a weight-average molecular weight ($\bar{M}w$) of greater than 7000. ^{1}H-NMR, ^{13}C NMR and IR spectra of the PBM obtained in this report completely agreed with those of an authentic sample.[4] It was also confirmed that BM was not polymerized to produce PBM without lipase under these conditions, indicating that the lipase catalyzed the polymerization of BM. Table 1 shows the typical ring-opening polymerization of BM by both the enzymatic method and the chemical method.

It was confirmed that polymerization occurred with both

porcine pancreatic lipase (PP) and microbial origin lipase, Novozyme 435 (Novo). No significant difference between the two enzymes was observed with respect to the molecular weight of the polymer. However, both the monomer conversion and the yield of PBM using PP were higher than that using Novo. During the lipase-catalyzed polymerization of BM using PP and Novo, no significant formation of BM oligomer was detected by GPC. From the measurements of $\overline{M}w$ and conversion with time, the $\overline{M}w$ of the resultant polymer by PP reached the highest value after 24 h, and this $\overline{M}w$ remained the same. On the other hand, the conversion was gradually increased with the reaction time.

It was reported by Guerin *et al.* that the optical rotation of optically active (-)-PBM with a molecular weight of 6000, which was obtained from L-aspartic acid, was $[\alpha]_D^{25}$ -5.0° (c=1, CH_2Cl_2).[12] When compared to the optically active (-)-PBM, the optical activity of PBM obtained by the lipase-catalyzed polymerization was low (entries 2 and 9 in Table 1). However, PBM obtained by Novo showed a slightly higher optical activity than that obtained by PP. Further analysis is now under study.

The addition of an organic solvent, such as isooctane and heptane, did not significantly affect the molecular weight and molecular weight dispersion of the resultant polymer.

Compared to the chemical polymerization of BM using betaine (entries 11 and 12 in Table 1), the lipase-catalyzed ring-opening polymerization of BM showed similar molecular weight ($\overline{M}n$). However, the lipase-catalyzed polymerization of BM tended to occur more quickly.

The benzyl group of PBM was readily removed by catalytic hydrogenation using Pd/C in the presence of cyclohexene or hydrogen gas to yield poly(β-D,L-malic acid) almost quantitatively.

In conclusion, it was found that high-molecular weight poly(β-malic acid) was prepared by the lipase-catalyzed ring-opening polymerization of BM using lipase followed by subsequent debenzylation.

References and Notes

1 F. Kawai, *Adv. Biochem. Eng./Biotech.*, **52**, 151 (1995).
2 G. Swift, *Polym. Degrad. Stabil.*, **45**, 215 (1994).
3 T. Ouchi and A. Fujino, *Makromol. Chem.*, **190**, 1523 (1989).
4 Y. Abe, S. Matsumura, and K. Imai, *J. Jpn. Oil Chem. Soc.*, **35**, 937 (1986).
5 M. Vert and R.W. Lenz, *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)*, **20**, 608 (1979).
6 S.C. Arnold and R.W. Lenz, *Makromol. Chem., Macromol. Symp.*, **6**, 285 (1986).
7 H. Uyama and S. Kobayashi, *Chem. Lett.*, **1993**, 1149.
8 R.T. MacDonald, S.K. Pulapura, Y.Y. Svirkin, R.J. Gross, D.L. Kaplan, J. Akkara, G. Swift, and S. Wolk, *Macromolecules*, **28**, 73 (1995).
9 Enzymes : Porcine pancreatic lipase (PP, 41 U/mg protein, according to the supplier) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Novozyme 435 (triacylglycerol hydrolase + carboxylesterase) having 7000 PLU/g (propyl laurate units) was kindly supplied by Novo Nordisk A/S (Bagsvaerd, Denmark).
10 Benzyl β-D,L-malolactonate (BM) was prepared by the cyclization of benzyl hydrogen bromosuccinate which was prepared using the bromosuccinic anhydride obtained by the treatment of maleic anhydride with hydrogen bromide according to Refs. 4 and 5.
11 The number-averge molecular weight *($\overline{M}n$)*, weight-averge molecular weight *($\overline{M}w$)* and molecular weight dispersion *($\overline{M}w/\overline{M}n$)* were measured by a gel permeation chromatography (GPC) using GPC columns (Shodex 80M, Showa Denko Co., Ltd., Tokyo) with a reflective index detector. Chloroform was used as the eluent. The GPC system was calibrated with a polystyrene standard.
12 P. Guerin, M. Vert, C. Braud, and R.W. Lenz, *Polym. Bull.*, **14**, 187 (1985).