Immobilized Lipase Showing High Catalytic Activity toward Enzymatic Ring-Opening Polymerization of Macrolides

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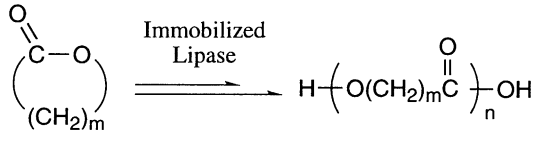
(Received October 18, 1995)

Immobilized *Pseudomonas fluorescens* lipase adsorbed on a Celite was used as new catalyst for enzymatic ring-opening polymerization of macrolides. Immobilization in the presence of a sugar or poly(ethylene glycol) greatly enhanced the catalytic activity.

Enzyme-catalyzed reactions in organic solvents have been increasingly popular for various synthetic purposes because of the high enzymatic specificities, in particular enantioselectivity and regioselectivity.[1] Since a powdery enzyme is often suspended directly in organic media, much amount of the enzyme is necessary owing to the heterogeneous reaction. To improve the enzyme activity in such media, various attempts, *e.g.*, immobilization on insoluble matrices,[2] complex formation with a lipid,[3] hybridization with a synthetic polymer,[4] have been investigated.

Recently, enzymatic syntheses of aliphatic polyesters have been extensively investigated.[5] Enzyme catalysis induced the polymerizations under mild reaction conditions to produce polyesters. Ring-opening polymerization of lactones, medium size lactones (6- and 7-membered) as well as macrocyclic lactones (12-, 13-, and 16-membered), have been polymerized by a lipase catalyst to the corresponding polyesters.[6-11] Especially, the macrolides with smaller strain in ring, then showing a lower anionic-polymerizability, were found to be polymerized much faster by lipase catalyst than ε-CL.[9,11] This is probably due to the stronger recognition of the macrolide by the enzyme.

Lipase-catalyzed polymerizations to polyesters often require a large amount of the enzyme[12] to achieve high molecular weight and/or high yields. This communication deals with an immobilized lipase showing high activity toward enzymatic ring-opening polymerization of macrolides (Eq. 1). By using the present catalyst, the enzyme amount can be dramatically reduced. A similar immobilized lipase has been reported,[13] which was well dispersed in an organic solvent and used for lactonization of 15-hydroxypentadecanoic acid in benzene. Until now, however, there has been no report on the Celite-immobilized lipase for the reduction of the catalyst amount.



(1)

m=10 : UDL
m=11 : DDL
m=14 : PDL

In this study, *Pseudomonas fluorescens* lipase (lipase PF, purchased from Biocatalysts) was employed, which was reported to induce the polymerization of lactones effectively.[6,9,11] Zaks and Klibanov demonstrated that enzyme activity in organic solvents was greatly dependent upon the pH of the aqueous solution from which the enzyme was lyophilized.[14] We first prepared immobilized enzymes by lyophilization of the aqueous enzyme solutions of different pH in the presence of a Celite (Hyflo Super-Cel).[15] A Celite is suitable as the support material of immobilized enzymes for use in organic solvents because of its low aquaphilicity.[16]

Table 1. Effect of the buffer pH in the immobilization of lipase PF on the enzymatic polymerization of DDL[a]

	Buffer				
Entry	pH	Salt	Conv./%[b]	M_n/x10^{-3}[b]	M_w/M_n[b]
1	4	Acetate	17	2.5	1.6
2	6	Phosphate	31	2.5	1.8
3	7	Phosphate	44	3.1	2.2
4	8	Phosphate	89	5.4	2.2
5	10	Carbonate	19	3.9	2.5

[a]Polymerization of DDL (0.60 mmol) using the immobilized lipase (30 mg) in bulk at 60 °C for 72 h. [b]Determined by GPC.

Table 1 shows results of 12-dodecanolide (13-membered lactone, DDL) polymerization catalyzed by the immobilized enzyme. The polymerization was performed in bulk at 60 °C for 72 h.[17] A maximum point of the monomer conversion is observed at pH 8. This agrees with the value at which lipase PF showed the highest activity in an aqueous solution.[18] Therefore, this pH 8 buffer is used in all subsequent experiments throughout the present study.

It was known that lyoprotectants enhance activity of some hydrolases in anhydrous solvents when present in enzyme-containing aqueous solution prior to lyophilization.[12,19] Typical lyoprotectants are sugars and water-soluble polymers. The enhanced activity is believed to be based on the ability of the lyoprotectants to alleviate reversible denaturation of enzymes during lyophilization.[19]

To further improve the catalytic activity of the present immobilized lipase, lyoprotectants in the same amount of lipase PF were added before the lyophilization. Here, cellobiose, glucose, mannitol, sucrose, and poly(ethylene glycol) (PEG, molecular weight = 2000) were used as lyoprotectants. The activity of the present immobilized lipase was evaluated in the polymerization of DDL for 24 h (Table 2). All the lyoprotectants enhanced the activity (entries 1-5), in comparison with the reactions without the lyoprotectant (entry 6); the monomer conversion as well as the polymer molecular weight increased by using the lyoprotectant. Among the lyoprotectants examined, sucrose gave the highest catalytic activity (entry 4).

The immobilized enzyme used for entries 1-5 contains 1.2-1.5 mg of the net lipase PF (~1 weight% for the monomer). For comparison, the polymerization using 1.5 mg of the enzyme

Table 2. Effect of lyoprotectants in the immobilization[a] of lipase PF on the enzymatic polymerization of DDL[b]

Entry	Lyoprotectant	Conv./%[c]	M_n/x10^{-3}[c]	M_w/M_n[c]
1	Cellobiose	86	4.0	2.0
2	Glucose	87	3.7	2.0
3	Mannitol	68	6.4	1.9
4	Sucrose	91	7.0	2.1
5	PEG[d]	89	4.0	1.9
6	---[e]	32	3.1	2.1

[a]Immobilization in the presence of the same amount of the lyoprotectant as lipase PF in the phosphate buffer (pH 8). [b]Polymerization of DDL (0.60 mmol) using the immobilized lipase (30 mg) in bulk at 60 ℃ for 24 h. [c]Determined by GPC. [d]Poly(ethylene glycol) of molecular weight = 2000. [e]Immobilization without the lyoprotectant.

powder was performed under the similar reaction conditions, resulting in the DDL conversion less than 1%. These data indicate that the immobilization technique dramatically improved the lipase activity for the polymerization of DDL.

The immobilized enzymes are also effective for the polymerization of other macrolides, 11-undecanolide (12-membered, UDL) and 15-pentadecanolide (16-membered, PDL). The polymerization of them was performed by using the catalyst prepared in the phosphate buffer of pH 8 at 60 ℃ for 72 h. UDL and PDL were consumed in 65 and 50%, respectively, yielding the polymer with molecular weight of 5600 and 1600, respectively. Under the similar conditions, the powdery enzyme (1.5 mg) did not induce their polymerization.

In conclusion, the immobilized lipase adsorbed on a Celite exhibited the high catalytic activity for the enzymatic ring-opening polymerization of macrolides. The addition of the lyoprotectants further improved the catalytic power. Further investigations including immobilization of other lipases exhibiting high activity as catalyst for polyester synthesis are under way in our laboratory.

This work was partly supported by Grants-in-Aid for Scientific Research (Nos. 06403026 and 07555592) from the Ministry of Education, Science, and Culture, Japan.

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