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SHORT COMMUNICATIONS

High-Performance Immobilized Lipase Catalyst for Polyester Synthesis

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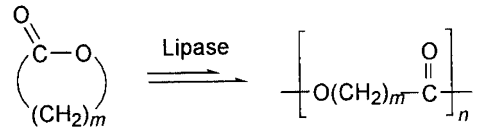
Lipase is an enzyme that catalyzes hydrolysis of fats (fatty acid triglycerides) in living cells. In a non-aqueous medium, on the other hand, lipase can act as catalyst for esterification and transesterification.[1] This characteristic property has been applied to lipase-catalyzed ring-opening polymerization and polycondensation under mild reaction conditions to biodegradable polyesters and polycarbonates.[2–9] Enantio- and regioselective polymerizations have been achieved *via* lipase catalysis to give functional polyesters, most of which can not be synthesized by conventional chemical catalysts.[10–15]

Candida antarctica lipase (lipase CA) immobilized on macroporous acrylic resin (Novozym 435) is industrially developed for modification of triglyceride oils. Previously, we first demonstrated highly efficient catalysis of Novozym 435 for polymerization of lactones;[16] a small amount of this enzyme (less than 1%) induced the polymerization of ε-caprolactone (ε-CL) and the polymerization rate using Novozym 435 was much larger than that by other commercially available lipases under the similar reaction conditions. Furthermore, Novozym 435 could be repeatedly used for the polymerization of ε-CL.[17] In the range of 5 cycles, the polymerization results hardly changed.

Activity of immobilized enzyme catalysts is well known to depend on their support properties such as hydrophilicity and porosity.[18] In this study, we have screened the enzyme support for development of the immobilized lipase catalyst for efficient production of polyesters.[19]

RESULTS AND DISCUSSION

In this study, four polymeric and one ceramic supports have been employed (Table I). All the supports are powdery in the diameter larger than 100 μm and have porous structures. There were no functional groups in the polymer supports (sample A–D) and the phenyl group was introduced on the surface of the pore in the ceramic support (sample E). The immobilization was carried out by mixing an aqueous solution of lipase CA and the support in a phosphate buffer of pH 7.0. The amount of protein fixed onto the support was calculated from the difference of protein content in the solution before and after the immobilization (Table II). The immobilized protein content scarcely depended on the support type except sample C, suggesting that a support with small poresize is not suitable for the immobilization of lipase CA. For reference, the amount of the immobilized protein of Novozym 435 was 175 mg g^{-1} support (data from supplier).



Scheme 1.

In order to examine the catalytic activity of the present immobilized lipases for the polyester synthesis, the polymerization of ε-CL was performed in toluene at 60 °C for 1 h (Scheme 1). The lipase immobilized on polypropylene (sample B) showed the highest activity. An immobilized lipase on porous polypropylene was reported to catalyze ester hydrolysis and esterification.[20–22] Samples D and E also induced the polymerization; however, there was no or little activity for the polymerization of ε-CL in samples A and C. Under the similar conditions, poly(ε-CL) was quantitatively formed in using Novozym 435 as catalyst. Afterwards, the catalytic activity of sample B for polyester production was examined.

We reported that macrolides showing less anionic polymerizability than ε-CL were more efficiently polymerized through lipase catalysis.[23] In the polymerization of 15-pentadecanolide (PDL), the catalytic activ-

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Table I. Supports for Immobilization of Lipase

Code	Material	Diameter[a] μm	Poresize[a] Å	Product	Supplier
A	Acryl Resin	300	170	HP2MG	Mitsubishi Chemical
B	Polypropylene	400	700	Accurel MP1000	Membrana
C	Polystyrene	250	57	SP825	Mitsubishi Chemical
D	Polystyrene	250	260	HP20	Mitsubishi Chemical
E	Ceramic	170	400	Toyonite 200-P	Toyo Denka Kogyo

[a] Data from supplier.

Table II. Ring-Opening Polymerization of ε-Caprolactone by Immobilized Lipase[a]

Code	Immobilized Protein[b] mg g^{-1} support	Conv.[c] %	M_n[d] $\times 10^{-3}$	M_W/M_n[d]
A	39	0		
B	46	88	7.2	1.5
C	5.2	14	0.6	1.4
D	34	51	4.1	1.3
E	37	57	3.1	1.2

[a]Polymerization of ε-caprolactone (0.20 g) using an immobilized lipase catalyst (20 mg) in anhydrous tolouene (0.60 mL) at 60 °C for 1 h. [b]Determined by bicinchoninic acid kit for protein determination (Sigma). [c]Determined by ^{1}H NMR. [d]Determined by SEC.

ity of sample B was compared with that of Novozym 435 (Figure 1). At all the temperatures examined, the monomer conversion in using sample B was larger than that by a commercially available immobilized lipase (Novozym 435), indicating the higher catalytic activity of sample B than Novozym 435. The number-average molecular weight (M_n) of the resulting polymer obtained by using sample B as catalyst at 60 °C was 1.9×10^4, which was close to that by Novozym 435 ($M_n = 2.1 \times 10^4$). These data clearly showed that the catalytic activity per the immobilized protein amount of sample B was much larger than that of Novozym 435.

Next, the catalytic activity of sample B for polycondensation was examined. Previously we demonstrated the high enzymatic reactivity of divinyl esters for synthesis of polyesters for the first time.[24] Here, the polycondensation of divinyl sebacate and 1,8-octanediol (each 2.0 mmol) was carried out using the lipase catalyst (50 mg) in anhydrous toluene (2.0 mL) at 75 °C (Scheme 2). In using sample B as catalyst, the monomer was completely disappeared after 1 h, and M_n of the polymer reached 1.4×10^4 after 24 h, which was larger than that by using Novozym 435 ($M_n = 1.0 \times 10^4$) under the similar reaction conditions. These data indicate that the present immobilized lipase also showed a higher catalytic activity for the polycondensation to polyesters than Novozym 435.

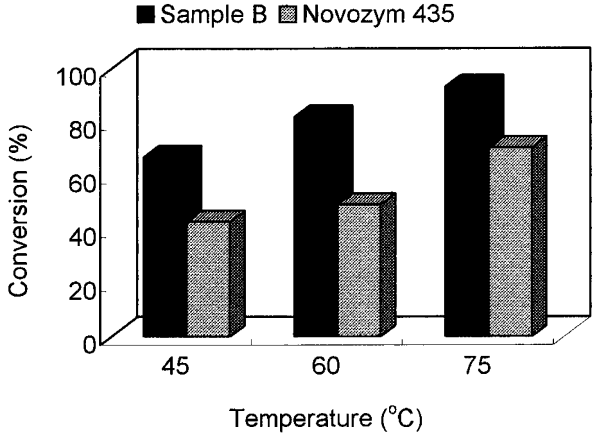
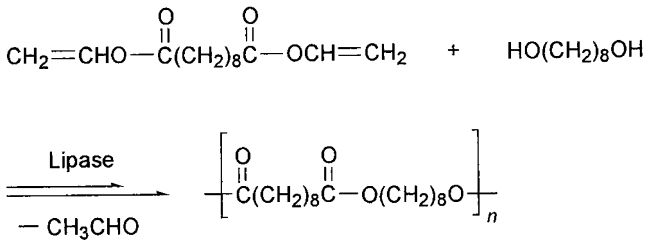


Figure 1. Comparison of catalytic activity between immobilized lipase on polypropylene (sample B) and Novozym 435 in the polymerization of PDL. The polymerization of PDL (0.20 g) was performed using an immobilized lipase catalyst (10 mg) at different temperature in anhydrous toluene (0.60 mL) for 30 min.



Scheme 2.

CONCLUSIONS

Various supports were screened for immobilization of lipase to develop biocatalyts showing high catalytic activity for production of aliphatic polyesters. Among them, porous polypropylene was found to be a good support for immobilization of *Candida antarctica* lipase. The immobilized lipase on polypropylene more efficiently catalyzed the ring-opening polymerization of 15-pentadecanolide and the polycondensation of divinyl sebacate and 1,8-octanediol than Novozym 435. Further studies on development of new immobilized biocatalysts for enzymatic polymerizations are under way in our laboratory.

EXPERIMENTAL

Lipase CA aqueous solution and Novozym 435 were kindly donated by Novozymes Japan Ltd. Supports of acryl resin and polystyrene were gifts from Mitsubishi Chemical Co. Ceramic and polypropylene supports were kindly donated by Toyo Denka Kogyo Co. and Membrana GmbH, respectively. These lipases and supports were used as received. Liquid monomers and toluene were commercially available and stored over freshly activated type 4 molecular sieves.

Immobilization of lipase on support was performed as follows. At first, support was washed with ethanol, an equivolume mixture of ethanol and water, and finally water. The washed support (1.0 g) and lipase CA aqueous solution given by the supplier (2.0 mL) were added to a phosphate buffer of pH 7.0 (19 mL). The mixture was gently stirred at 4 °C for 4 h. The support was separated by filtration and lyophilized. The protein content of the filtrate was determined by bicinchoninic acid kit for protein determination (Sigma).

Polymerization of lactone was carried out as follows. A mixture of lactone, immobilized lipase, and anhydrous toluene was placed in a dried test tube under argon. The mixture kept at desired temperature for 0.5 or 1 h under gentle stirring. After separation of the immobilized lipase by filtration, the solvent of the filtrate was removed under reduced and the residue was dried in vacuo.

SEC analysis was carried out by using a Tosoh SC8020 apparatus equipped with refractive index (RI) detector at 40 °C under the following conditions: TSKgel G3000 H_{HR} or G4000 H_{HR} column and tetrahydrofuran or chloroform eluent at a flow rate of 1.0 mL min^{-1}. The calibration curves were obtained using polystyrene standards. ^{1}H NMR spectra were recorded on a Bruker DPX400 spectrometer.

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