Supplementary information

Substrate Imprinted Lipase Nanogel for One-step Synthesis of Chloramphenicol Palmitate

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Materials

Lipase from *Thermomyces lanuginosus* (Lipozyme TL 100L) was purchased from Novozyme. *N*-acryloxysuccinimide, ammonium persulfate, *N,N,N',N'*,-tetramethylethylenediamine, acrylamide,chloramphenicol, vinyl palmitate and para-nitrophenyl palmitate were purchased from Sigma-Aldrich. The TSK- Gel G2000 SW XL column was purchased from TOSOH (Tokyo, Japan). The ZORBAX Rx-SIL column (5μm, 4.6×250mm) was purchased from Agilent. The VP-ODS column (5 μm, 4.6×250mm) was purchased from SHIMADZU. Other chemicals were all of analytical grade.

Synthesis of lipase nanogel

The first step was the acryloylation, in which 6 mg of *N*-acryloxysuccinimide (NAS) in 0.8 mL of dimethyl sulfoxide (DMSO) was slowly added to 10 mL of acetic buffer (pH 5.0, 50 mM) containing 4 mg/mL of Lipozyme TL 100L. The mixture was incubated at 30 °C for 6 h. Unreacted reagents were removed by 48 h dialysis against acetic buffer (pH 5.0, 50 mM). The second step was the *in situ* polymerization, in which 0.5 g of acrylamide (AM) was added to the acryloylated lipase solution. After 30 min N_2 purging, *in situ* polymerization was initiated by adding 30 mg of ammonium persulfate (APS) and 25 μL of *N,N,N',N'*-tetramethylethylenediamine (TEMED). After 12 h reaction, the product solution was subjected to 48 h dialysis against acetic buffer (pH 5.0, 50 mM) at 4 °C to remove unreacted reagents.

Preparation of substrate imprinted lipase nanogel and free lipase

The substrate imprinting was carried out during lyophilization. In a typical experiment, 0.3 g of palmitic acid in 2 mLof acetone was slowly added to 75 mL of acetic buffer (50 mM, pH 5.0) solution containing lipase nanogel (4 mg/mL of protein in the solution) or native lipase (4 mg/mL of protein in the solution) under vigorous agitation. The solution was lyophilized to generate lipase nanogel or native lipase in the powder form. After washing for 3 times with petroleum ether having boiling point range of 60-90 °C, the imprinted nanogel was subjected to volatilization and then stored at 4 °C.

Lipase-catalyzed transesterification reaction between chloramphenicol and vinyl palmitate

The lipase-catalyzed transesterification reaction between chloramphenicol and vinyl palmitate was conducted in 2 mL of acetonitrile containing chloramphenicol (10 mg), enzyme catalyst (25 mg)

and different amounts of vinyl palmitate. The molar ratio of chloramphenicol to vinyl palmitate was varied from 1:1 to1:15. The mixture was shaken for 48 h at 200 rpm at 20 °C, 30 °C or 40 °C, respectively. The product was purified by recrystallisation in ethnol. The structure of product was determined by ^{1}H-NMR and ^{13}C-NMR[1]. ^{1}H-NMR ($CDCl_3$, 600.17 MHz): 0.87 (t, $^{3}J_{HH}$=6.9 Hz, 3H), 1.25 (s, 24H), 1.62 (t, $^{3}J_{HH}$=7.6 Hz, 2H), 2.37 (t, $^{3}J_{HH}$ =7.9 Hz, 2H), 4.18-4.20 (m, 1H), 4.46-4.48 (m, 2H), 5.05 (d, $^{3}J_{HH}$=1.5 Hz, 1H), 5.76 (s, 1H), 6.90 (d, $^{3}J_{HH}$=8.9 Hz, 1H), 7.55 (d, $^{3}J_{HH}$=8.6 Hz, 2H), 8.19 (d, $^{3}J_{HH}$=8.6 Hz, 2H); ^{13}C-NMR ($CDCl_3$, 150 MHz): 14.2 (CH_3), 22.8(CH_2), 24.7(CH_2), 24.8(CH_2), 29.3-29.7 (8CH_2), 32.0(CH_2), 33.8(CH_2), 34.2(CH_2), 54.2 (CH), 62.4 (CH_2), 66.1(CH), 70.6(CH), 123.8 (2CH), 126.8(2CH), 147.1(C), 147.7 (C), 164.5 (C), 174.6 (C).

Lipase-catalyzed transesterification reaction between ascorbic acid and vinyl palmitate

The lipase-catalyzed transesterification reaction between ascorbic acid and vinyl palmitate was conducted in 5 mL of tert-butanol containing ascorbic acid (5 mg), enzyme catalyst (10 mg) and 40 mg of vinyl palmitate. The mixture was shaken for 72 h at 200 rpm at 50 °C. Then the yield of product was determined by high performance liquid chromatography (HPLC) analysis.

Assays

Protein concentration. The protein concentration was determined by the bicinchoninic acid (BCA) method using the protein assay kit purchased from Beyotime, China.

Size exclusion chromatography. Size exclusion chromatography was carried out using a TSK-GEL SW4000xL column (TOSHO) with fluorescence detector (RF-10AxL, SHIMADZU). Samples were eluted at a flow rate of 0.5 mL/min with 0.1 M sodium phosphate buffer (pH 6.7) containing 0.1 M Na_2SO_4 and 0.05 % NaN_3 as the mobile phase. The elution profiles were recorded by the fluorescence detector with an excitation wavelength at 280 nm and an emission wavelength at 330 nm. The protein encapsulation yield was measured in terms of the peak area of the encapsulated lipase over the total area of protein fractions.

High performance liquid chromatography(HPLC) analysis. The yield and purity of chloramphenicol palmitate were determined by high performance liquid chromatography using Agilent ZORBAX Rx-SIL column (5 μm, 4.6×250mm) with a SHIMADSU SPD-10AVP UV-vis detector at 254 nm[2]. In the assay, 20 μL of sample was injected and eluted at 1 mL/min with 4.8 vol % of 2-propanol in hexane. For the calculation of the purity and yield, calibration curves were done with standard solution of chloramphenicol and chloramphenicol palmitate. The yield of ascorbic acid palmitate was determined using Shimadzu VP-ODS column (5 μm, 4.6×250mm) with a SHIMADSU SPD-10AVP UV-vis detector at 252 nm[3]. In the assay, 20 μL of sample was injected and eluted at a flow rate of 1 mL/min with methanol containing 15% of acetic buffer (pH 2.6). For the calculation of the yield, calibration curves were done with the standard solution of ascorbic acid.

Hydrolytic activity. The hydrolytic activity of lipase nanogel and native lipase were determined using para-nitrophenyl palmitate as the substrate. Para-nitrophenyl palmitate was first dissolved in acetone and then added to the phosphate buffer (50 mM, pH 7.0) containing 1.25% (w/v) of Triton X-100. The reaction was started by adding 50 μL of enzyme solution to 950 μL of substrate

solution and detected at 348 nm.

Transesterification activity. Different preparations of lipase nanogel with the same protein content (0.5 mg protein) were mixed with 0.5 mL of n-heptane solution containing 10 mM para-nitrophenyl palmitate. 30μL of ethanol was then added to the above mixture to start the reaction, followed by incubation at 40°C at 200 rpm for 1 h. Then 25 μL of the clear supernatant was taken out and immediately mixed with 1 mL of 0.1 M NaOH. The para-nitrophenol liberated was extracted by the aqueous alkaline phase, and detected at 410 nm using a UV–visible spectrophotometer (Shimadzu UV2550).

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The TEM images of the imprinted and non-imprinted enzyme nanogels were recorded using a Hitachi H-7650B high-resolution TEM. The sample was diluted in distilled water to a concentration of 0.1 mg/mL, and then dropped on the surface of a carbon-coated grid. After removing the excess, negative staining was carried out by adding 2% sodium phosphotungstate water solution (pH 7.0). The sample was then subjected to TEM measurement. The powder of lipase nanogel was placed on the SEM conductive tape and directly visualized under SEM.

References

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