An Immobilized Lipase Microfluidic Reactor for Enantioselective Hydrolysis of Ester

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Enantioselective enzymatic hydrolysis of racemic mixture is an effective method to obtain pure enantiomer. In this report, an immobilized lipase microfluidic reactor was fabricated and applied to enantioselective hydrolysis of racemic 1-phenylethyl acetate to obtain (*R*)-1-phenylethanol. This immobilized lipase microfluidic reactor consumed trace amount of enzyme, showed identical performance compared to free enzyme in batch, and good stability that can be recycled for at least eight times without obvious loss of activity.

Production of chiral precursors or intermediates is now in high demand in the pharmaceutical and agricultural industry.[1] Great efforts have been made to develop different methods to produce chiral compounds. Among various methods for obtaining chiral compounds, enzyme-catalyzed enantioselective resolution has been considered to be useful, because enzymes show high catalytic efficiency under mild conditions, and high chemo-, regio-, and enantioselectivities.[2,3] Generally, enzymes can be used in free or immobilized modes. Compared with a free enzyme in solution, enzyme immobilized on the surface of a suitable carrier material is more stable and convenient for reuse.[4,5]

Microfluidic reactors are a new reaction platform which are characterized by efficient heat transfer and mass transport, large surface-to-volume ratio and microlitre-scale consumption of reagent and sample, and have shown advantages such as speed, throughput, and controllability in the field of chemical synthesis in some cases.[6,7] Compare with glass capillary, a microfluidic reactor is more flexible to design and easy to be integrated with other operation units such as micro-mixer, micro-extractor, micro-heater, etc. Recently, free enzyme-catalyzed asymmetric reaction and separation of (*S*)-ibuprofen on microfluidic reactors has been carried out.[8,9] To our knowledge, however, immobilized enzyme-catalyzed racemate resolution in microfluidic reactors has not been reported yet. Here, we fabricated an immobilized enzymatic microfluidic reactor made of glass, and applied it to enantioselective resolution of ester. Immobilized lipase from *Burkholderia cepacia* (BCL) catalytic enantioselective hydrolysis of racemic 1-phenylethyl acetate was used as a model reaction, seen in Figure 1a. In this reaction, the lipase selectively hydrolyzes the (*R*)-1-phenylethyl acetate and produces (*R*)-1-phenylethanol, acetic acid and unreacted (*S*)-1-phenylethyl acetate.

Figure 1b shows the setup of the microfluidic reactor system used in this work. The modification of the microfluidic reactor channel and the introduction of the substrate were performed by a syringe pump (Harvard Apparatus, USA). Figure 1c displays the microfluidic reactor. The reactor was fabricated in borosilicate glass following standard photolithographic and wet chemical techniques.[10] A 41-cm-long serpentine reaction

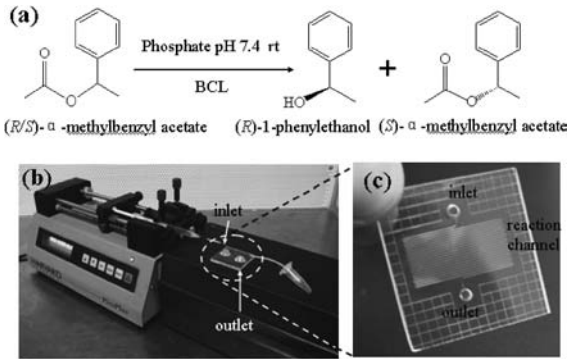

Figure 1. Immobilized lipase microfluidic reactor. (a) Schematic illustrating the reaction of BCL catalytic hydrolysis of *rac*-1-phenylethyl acetate, (b) setup of the microfluidic reactor system, (c) photograph of the microfluidic reactor.

channel (200 μm wide, 25 μm deep) was fabricated in a 3 cm × 2.5 cm × 2.3 mm glass chip.

Compared with a conventional batch reactor, microfluidic reactors are more effective in control of reaction condition, such as temperature and pH.[6] For the model reaction used in this work, the reaction medium is usually maintained near neutral,[11,12] because pH fluctuation could affect the efficiency of hydrolysis by affecting the activity of enzyme. In conventional batch reaction, to maintain the pH of the reaction medium, low concentration of NaOH was added to the reaction medium by an automatic titration to decrease the effect of the acetic acid produced.[12] By using the immobilized enzymatic microfluidic reactor, the products and unreacted reactant were continuously pushed out from the reaction channel, the following reactant entered the reaction channel was little affected by the acetic acid produced in prophase.

Lipase was covalently immobilized on the wall of microfluidic reactor according to the method reported previously.[13] Firstly, the channel was silanized by 10% (V/V) 3-aminopropyltrimethoxysilane (APTS) in methanol. Then 10% (V/V) glutaraldehyde dissolved in phosphate buffer (20 mM, pH 8.0) was introduced into the channel for 3 h at room temperature. Finally, enzyme solution was applied to the channel with low velocity of flow for approximately 24 h. Then the reactor channel was washed with phosphate buffer (20 mM, pH 7.4) and stored at 4 °C until use. Here, glutaraldehyde not only connected with lipase, but also offered a space arm with five carbon atoms, which may contributed to keep the reactivity of the lipase. The content of the lipase immobilized on the wall of the microfluidic reactor channel was measured using a micro-BCA method.[14] Approximately 14 μg of lipase was covalently immobilized on the channel wall.

Diluted racemic ester (10 mM, 10 μL) was introduced into the channel with different velocity of flow controlled by the

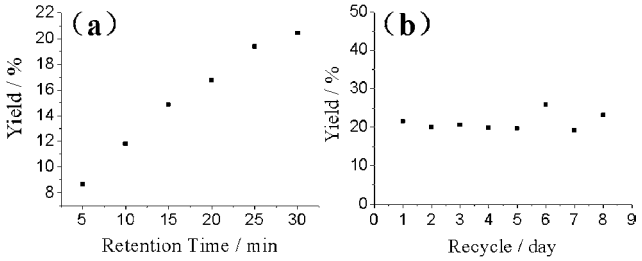

Figure 2. The performance of the immobilized lipase microfluidic reactor. (a) The effect of the retention time on the continuous hydrolysis of the *rac*-1-phenylethyl acetate, (b) recycle result of the immobilized lipase on the microfluidic reactor for enantioselective hydrolysis of *rac*-1-phenylethyl acetate with a retention time of 30 min. Chromatographic analysis conditions: column: 250 × 4.6 mm I.D. packed with 5 μm Kromasil-ODS, flow rate: 1 mL/min, detection wavelength: 214 nm.

syringe pump. Product was collected in a tube. The yield and enantiomeric excess (ee %) of (*R*)-1-phenylethanol were tested by analytical high-performance liquid chromatography (HPLC).

The relationship of the retention time and product yield was examined. As can be seen from Figure 2a, the product can be detected even if the reactant is only reserved in the channel for 5 min and with the increase of retention time, the yield of (*R*)-1-phenylethanol was increased. The result demonstrated that the immobilized lipase microfluidic reactor was effective for the model reaction.

The stability of the immobilized lipase microfluidic reactor was evaluated. Figure 2b shows the reusability of this microfluidic reactor used for enantioselective hydrolysis of *rac*-1-phenylethyl acetate. When each reaction was over, the microfluidic reactor was filled with phosphate buffer and kept at 4 °C over night. During 8 recycle trials, the yield was distributed between 19–25%, and after 8 cycles the reaction performance of the immobilized lipase did not decrease obviously. Actually, no apparent loss of activity of the immobilized lipase on microfluidic reactor was observed even after 3 months. The stable conformation of lipase after immobilization and greatly reduced mechanical damage without stirring might prolong the life of lipase immobilized in the microfluidic reactor.

Free enzymatic hydrolysis of *rac*-1-phenylethyl acetate in batch was carried out keeping the reaction time and the ratio of ester and lipase identical with that of the microfluidic reactor. Results are summarized in Table 1. The yield of (*R*)-1-phenylethanol by using free lipase in a flask (lot number 2) was close to that obtained from microfluidic reactor. These results showed that the activity of lipase was not influenced through the procedure of immobilization. Compared with free lipase reaction, the manipulation on a microfluidic reactor was simpler. The process such as stirring and quenching the reaction were not necessary in the immobilized lipase microfluidic reactor. Furthermore, the microfluidic immobilized lipase reactor can be used repeatedly.

In conclusion, the immobilized enzymatic microfluidic reactor for enantioselective hydrolysis of ester was first demonstrated. The immobilized lipase microfluidic reactor provided a remarkably simplified reaction system and lipase did not need prepurification before immobilization. Reaction conditions could be optimized rapidly with microliters of reagent. Furthermore, the immobilized lipase microfluidic reactor displays good stability. The results show that the immobilized enzyme microfluidic reactor has the potential of being a simple and convenient experimental exploration for substrate screening and enzyme evaluation.

Table 1. Comparison of the immobilized lipase and free lipase[a,b]

Lot number	Enzyme	Enzyme dosage /μg	Ester dosage /μg	Yield /%	Product /ee %
1	Immobilized lipase	14	33	20	95
2	Free lipase	1400	3284	18	91

[a]The data are mean values from duplicate experiments. [b]Conditions for yield analysis as in Figure 2. Chromatographic conditions for enantiomeric excess: column: 250 × 4.6 mm I.D. packed with cellulose tris(3,5-dimethylphenyl carbamate) coated chiral stationary phase; mobile phase: *n*-hexane/2-propanol 98/2 (v/v); flow rate: 1 mL/min; detection wavelength: 254 nm.

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