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Lipase from *pseudomonas cepacia* immobilized into ZIF-8 as bio-catalyst for enantioselective hydrolysis and transesterification

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ABSTRACT

Enzyme immobilization in MOFs offers retained enzyme integrity and activity, enhanced stability, and reduced leaching. In this work, *Pseudomonas cepacia* lipase (PCL) was successfully immobilized into the Zeolitic imidazolate framework-8 (ZIF-8) by physical adsorption. The amounts of PCL in the immobilized enzyme (PCL@ZIF-8) was determined to be 16.7 % (200.5 mg PCL/g ZIF-8). Furthermore, the immobilized enzyme was applied as efficient bio-catalyst for enantioselective hydrolysis of 2-phenylpropionic acid (2-PPA) ester enantiomers and enantioselective transesterification of 1-phenylethanol enantiomers. The enzymatic activity of the immobilized enzyme was three times more than that of free PCL in enantioselective hydrolysis system, and the enantiomeric excess was maintained above 99 %. There was no significant difference in enzyme activity between immobilized PCL and free PCL in transesterification system. In addition, the immobilized enzyme showed good reusability in both hydrolysis (30.57 % of initial activity, 4 cycle) and transesterification reaction systems (64.38 % of initial activity, 6 cycle).

1. Introduction

Chiral drugs play an important role in the global pharmaceutical industry. The pharmacological actions of chiral drug are achieved by a strict chiral matching between the drug enantiomer and the macromolecule in the human body. Thus, there are significant differences in the absorption, distribution, pharmacological activity, metabolic processes and toxicity of different enantiomers of chiral drugs. In most cases, these differences result in one enantiomer being efficacious, while another enantiomer is ineffective or even has toxic side effects [1–3]. The resolution of enantiomers is a crucial work for improving the activity of chiral drugs and reducing the toxicity and metabolic burden of the human body [4].

2-Arylpropionic acid derivatives are a class of important non-steroidal anti-inflammatory drugs (NSAIDs), which have favorable analgesic, antipyretic and anti-inflammatory effects [5]. In general, the activity of (S)-enantiomer is much higher than that of (R)-enantiomer. For example, the anti-inflammatory and analgesic effect of (S)-ibuprofen is more than 100 times in comparison with (R)-ibuprofen. Likewise, (S)-flubuprofen is 30 times higher than (R)-flubuprofen [6]. Therefore, the optically pure drugs are preferred in clinical treatment. 2-PPA enantiomers are common pharmaceutical intermediate for the synthesis of various 2-arylpropionic acid derivatives. Therefore, the acquisition of optically pure 2-PPA enantiomer is significant in pharmaceutical field. Chiral alcohols, which have wide application value in medicine, are an important class of synthetic precursors with stable physicochemical properties. Chiral alcohol can be used to synthesize a variety of drugs for the treatment of diseases such as cardiovascular and hypertension [7]. 1-Phenylethanol is an important optically active substance. (S)-1-phenylethanol can be used to synthesize sertraline for the treatment of depression, as well as drugs for asthma and immune enhancement. (R)-1-phenylethanol can be used to synthesize drugs that inhibit cholesterol absorption [8]. Therefore, the preparation of a single 1-phenylethanol enantiomer is of great significance in the pharmaceutical industry. In recent years, researchers have obtained single enantiomers by a variety of separation methods [9]. The enzymatic kinetic resolution has emerged as an attractive method [10].

Lipases (triacylglycerol acyl ester hydrolases, EC 3.1.1.3) have been universally applied enzymes for the resolution of chiral drugs, which has the characteristics of wide substrate specificity, high regioselectivity, and enantioselectivity [11]. A structural feature that is common to most lipases is the presence of a so-called “lid”, composed of one or even two α-helix peptides, that covers the active site of the lipase. This polypeptide chain may fully isolate the lipase from the reaction medium

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[12–14]. Lipase from pseudomonas cepacia has molecular weight ranging between 28 and 33 kDa and average molecular diameter of 4−5 nm. Lipases from different groups have distincts properties. PCL has an optimal temperature at 50 ℃ and optimal pH of 7.0. Compared with other lipase, PCL is stable in organic solvents, with some exception stimulation or inhibition [15]. PCL is widely applied in enantioselctive hydrolysis, esterification and transesterification reaction due to high substrate specificity and enantioselectivity [16]. Nevertheless, PCL mainly exists in the form of enzyme powder in the market, which limits their repeated use in industrial processes. Generally, immobilization of lipase is one of the effective strategies to solve these problems, such as high cost, difficult recovery and low stability [17]. Immobilizing enzymes on a solid support can improve enzyme stability as well as make them ease of separation and recovery while maintaining selectivity and activity [18]. Up to now, immobilization techniques have been developed maturely, such as physical adsorption [19], covalent linkage [20], pore entrapment [21] and crosslinking [22]. In addition to immobilization techniques, the choice of solid supports is also extremely important for the immobilization of enzymes.

Metal-organic frameworks (MOFs), a class of novel porous materials, which are consist of organic ligands and inorganic metal nodes through coordination bonds [23]. Because of the diversity of organic ligands and metal ions as well as the ways of their combination, MOFs materials have a variety of types, which have been counted to more than 20,000 kinds. MOFs have been given increasing attention due to their diverse crystal structure, controllable pore size, high porosity and specific surface area [24]. According to functional requirements, MOFs can be practically applied in many aspects through post-modification and structural adjustment of the pores and structures, including adsorption separation [25], photoelectric induction [26], biotechnology [27] and others. Immobilization of enzymes on less uniform solid support typically results in low protein loading efficiency [28], low stability at elevated temperatures, and enzymatic leaching. MOFs have good adjustability, crystallinity and uniformity. Therefore, MOFs may be superior to other commonly used porous materials for immobilizing proteins and enzymes, such as sol-gel, zeolite and mesoporous silicon-ica supports. Zeolitic imidazolate frameworks (ZIF) are a class of promising supports for enzyme immobilization in various MOFs [29], because of their easy synthesis, excellent stability and negligible cytotoxicity [30]. In the past few years, MOFs have been used as a popular support for immobilization of enzymes, and excellent results have been achieved in catalytic applications [31]. However, there is rarely report on enantioselective catalyzed resolution of chiral enantiomers by using the immobilized enzymes as bio-catalyst.

In this work, *Pseudomonas cepacia* lipase was immobilized into ZIF-8 (hereafter denoted PCL@ ZIF-8) by using physical adsorption. The structure of ZIF-8 and PCL@ ZIF-8 was characterized by powder X-ray diffraction spectrometry (PXRD). Besides, the ZIF-8 and PCL@ ZIF-8 were further characterized by Fourier Transform Infrared Spectroscopy (FTIR), N_2 adsorption-desorption methods, and thermogravimetric analysis (TGA) to confirm the immobilization of PCL into ZIF-8. PCL@ZIF-8 was used as bio-catalyst for enantioselective hydrolysis of 2-PPA ester enantiomers and transesterification of 1-phenylethanol enantiomers to evaluate the catalytic activity and selectivity. In addition, the stability of immobilized enzyme was evaluated by investigating reusability.

2. Materials and methods

2.1. Materials

Lipase from *Pseudomonas cepacia* (PCL, 100,000 U/g) was acquired from Amano Enzyme Inc. (Nagoya, Japan). (R, S)-2-PPA (purity > 99 %), 1-phenylethanol (purity > 98 %) and 2-Methylimidazole (purity > 98 %) were obtained from Adamas Reagent Co., Ltd. (Shanghai, China). (R, S)-2-PPA esters were prepared in the laboratory. Hydroxypropyl-β-cyclodextrin (HP-β-CD) (purity > 99 %) was bought from Shandong New Fine Chemical Co., Ltd. (Shandong, China). Phosphoric acid, acetic acid, triethylamine and disodium hydrogen phosphate dodecahydrate were purchased from Huihong Reagent Co., Ltd. (Shanghai, China). Zinc nitrate hexahydrate ($Zn(NO_3)_2{\bullet}6H_2O$) was bought from Shanghai Aladdin Bio-Chem Technology Co. Ltd. (Shanghai, China). *N*, *N*-dimethylformamide (DMF) (purity > 99.5 %) was acquired from Shanghai Titan Scientific Co., Ltd (Shanghai, China). Sodium dodecyl sulfate (SDS) (purity > 99 %), p-nitrophenyl palmitate (p-NPP) (purity > 98 %), p-nitrophenol (p-NP) (purity > 99 %) were purchased from Sigma-Aldrich (USA). Solvent for chromatography was of HPLC grade. All other chemicals were of analytical-reagent grade.

2.2. HPLC analysis

The analysis of (R)-2-PPA and (S)-2-PPA were performed by high performance liquid chromatography (HPLC) composed of binary pump system (Waters 1525). An Inertsil ODS-3 column (250 mm × 4.6 mm, 5 μm) was employed. The wavelength of UV/visible detector (Waters 2489, U. S. A) was 230 nm. The mobile phase was consisting of methanol and aqueous solution (containing 25 mmol/L HP-β-CD and 0.5 % acetic acid) at the volume ratio of 20:80 (adjusted to pH = 4.0 with triethylamine) [32]. The flow rate of the mobile phase and the column temperature were maintained at 1.0 mL/min and 298 K, respectively. The injection volume of each sample was 10 μL. The retention time of (R)-2-PPA and (S)-2-PPA were 24.10 and 27.17 min, respectively. The (R, S)-2-PPA esters were monitored by HPLC, and the mobile phase was composed of acetonitrile and water at the volume ratio of 70:30. Other chromatographic conditions are the same as (R, S)-2-PPA.

In the enantioselective hydrolysis of (R, S)-2-PPA ester, the enantiomeric excess of (S)-2-PPA (ee_p) and conversion rate (c_R) of (R)-2-PPA were calculated as follows:

$$ee_P = \frac{[PPA_S] - [PPA_R]}{[PPA_S] + [PPA_R]} \times 100\% \quad (1)$$

$$c_R = \frac{[PPA_R]}{[S_R]_0} \times 100\% \quad (2)$$

where $[PPA_S]$ and $[PPA_R]$ are the concentrations of (S)-2-PPA and (R)-2-PPA after the reaction, respectively; $[S_R]_o$ represents initial concentration of (S)-2-PPA ester.

The remaining 1-phenylethanol enantiomers in reaction system were analyzed by HPLC employing a Waters e2695 series apparatus. The column was a Chiralcel® OJ-RH column (250 mm × 4.6 mm, 5 μm, Japan). The wavelength of photodiode array detector (Waters 2998) was 210 nm, and the column temperature was 298 K. The mobile phase was a 20:80 (v/v) mixture of acetonitrile and water. The flow rate was 0.5 mL/min, and the injection volume was set at 10 μL. The retention time of (S)-1-phenylethanol was less than that of (R)-1-phenylethanol.

In the enantioselective transesterification of 1-phenylethanol enantiomers, the enantiomeric excess of the product (ee_p) was determined by the enantiomeric excess of the substrate (ee_s) and total conversion rate (c).

$$ee_P = \left(\frac{ee_S}{c} - ee_S\right) \times 100\% \quad (3)$$

where

$$ee_S = \frac{[A_{(S)}] - [A_{(R)}]}{[A_{(S)}] + [A_{(R)}]} \times 100\% \quad (4)$$

$$c = \left(1 - \frac{[A_{(S)}] + [A_{(R)}]}{[A_{(S)}]_0 + [A_{(R)}]_0}\right) \times 100\% \quad (5)$$

where $[A_{(S)}]$ and $[A_{(R)}]$ represent the substrate concentration of (S)-1-phenylethanol and (R)-1-phenylethanol after reaction, respectively;

$[A_{(S)}]_0$ and $[A_{(R)}]_0$ represent the initial concentration of (S)-1-phenylethanol and (R)-1-phenylethanol, respectively.

2.3. Preparation and characterization of the immobilized lipase

Preparation of ZIF-8: The ZIF-8 was prepared according to the literature [33]. In brief, 2-methylimidazole (7.5 mmol) and Zn $(NO_3)_2$•$6H_2O$ (2.5 mmol) were mixed into the DMF (600 mL) solution in the round-bottomed flask. When the mixture turns out a homogeneous phase, the round-bottomed flask was placed in an oil bath at 413 K one day. After cooled to ambient temperature, the mixture was rinsed three times with methanol (3 × 100 mL), then brownish-black solid product was obtained after drying 12 h in a vacuum oven at 353 K. The structure of ZIF-8 was characterized by powder X-ray diffraction spectrometry (PXRD) and scanning electron microscope (SEM).

Immobilization of lipase: 400 mg ZIF-8 and 160 mg lipase PS were dispersed in 80 mL deionized water to form a mixture. The mixture was put in the Thermostatic oscillator at 298 K for 24 h. Lipase PS immobilized into ZIF-8 (PCL@ZIF-8) was rinsed with deionized water three times. Finally, the PCL@ZIF-8 after washing was dried by freeze dryer for 12 h. The chemical composition of PCL@ZIF-8 was analyzed by the Fourier Transform Infrared Spectroscopy (FTIR).

2.4. Assay of enzyme activity and protein assay

The enzyme activities in the immobilization process were determined with the substrate of p-nitrophenyl palmitate (p-NPP). In 50 mM sodium phosphate at pH 7.5 and 25 ℃, the concentration of p-nitrophenol (p-NP) produced by lipase catalyzed hydrolysis of 0.2 mM p-NPP was determined by UV spectrophotometer at 410 nm. One international unit of activity (U) was defined as the amount of enzyme that produce 1 μmol NP per minute under the conditions described previously. In order to facilitate the investigation of the influence of variables on the activity of immobilized enzymes. Take the highest value of enzyme activity in the same group of experiments as 100 %.

The immobilization efficiency was deuced by BCA (bicinchoninic acid) method, using bovine serum albumin (BSA) as standard. Analyze the protein concentration in the enzyme solution with microplate reader. The immobilized yield of lipase was indirectly determined by comparing the protein concentration difference between the enzyme solution provided before immobilization and the filtrate separated after immobilization. The immobilized yield (IY) was described by Eq. (6).

$$IY(\%) = \frac{C_0 - C_1}{C_0} \times 100\% \quad (6)$$

C_0 and C_1 are the protein concentration of lipase solution initially and finally, respectively.

2.5. Catalytic performances study

Preparation of (R, S)-2-PPA ester: (R, S)-2-PPA and isobutanol with equivalent molar were dissolved in toluene for esterification reactions, with *p*-toluenesulfonic acid as catalyst. After stirring for one night at 383 K, (R, S)-2-PPA esters were synthesized. The saturated $NaHCO_3$ solution (3 × 40 mL) was added to remove the excess acid in mixture solution. Then, distilled water (3 × 40 mL) was used to clean above (R, S)-2-PPA esters to neutral. The anhydrous $MgSO_4$ was applied to dry separated organic phase for 12 h, and then the solid was filtered. Finally, the toluene was removed by evaporation with lower pressure, and the yellow liquid product was obtained, that is (R, S)-2-PPA ester. In the end, HPLC analysis was used to confirm the synthesized products.

Enzymatic hydrolysis: Enzymatic hydrolysis reaction was performed in a 10 mL glass tube with a screw seal. The hybrid media was composed as follows: 2 mL of phosphate buffer solution (PBS, 0.1 mol/L, pH = 6.0) as reaction medium, 2.5 mg/mL free PCL or 12.5 mg/mL PCL@ZIF-8 as bio-catalyst and 10 mmol/L (R, S)-2-PPA ester as the reaction substrate. Both free PCL and PCL@ZIF-8 are identical in enzyme content. The temperature and stirring speed of thermostatic reactor (IKA®RCT CV S025, Werke GmbH & Co. KG) were maintained at 328 K and 400 rpm, respectively. After a certain period of reaction, the reaction mixture was filtered to obtain a sample. HPLC detected the concentrations of products. Kinetic resolution of 2-PPA enantiomers by the enzymatic is displayed in Fig. 1.

Enzymatic transesterification: Enzymatic transesterification of 1-phenylethanol was carried out in a 25 mL glass tube with a screw seal. The hybrid media was composed as follows: 3 mL of hexane as reaction medium, 5 mg free PCL and 25 mg PCL@ZIF-8 as bio-catalyst, 20 mmol/L 1-phenylethanol and 100 mmol/L acetoxyethylene as the reaction substrate. The reaction temperature and agitation speed in thermostatic reactor were kept at 310 K and 500 rpm, respectively. The sample was obtained by filtering the enzyme out of the reaction solution. HPLC detected the products concentrations. The reaction mechanism of enzymatic transesterification was shown in Fig. 2.

2.6. Reusability of PCL@ZIF-8

The reusability of PCL@ZIF-8 was studied by reusing 4 times for enantioselective hydrolysis of (R, S)-2-PPA ester and 6 times for enantioselective transesterification of 1-phenylethanol. In the hydrolysis of (R, S)-2-PPA ester, the PCL@ZIF-8 was recovered by filtration and washed with 0.1 mol/L PBS (pH = 6.0) after every cycle. In the transesterification of 1-phenylethanol, the PCL@ZIF-8 was washed with hexane (3 × 30 mL) after every cycle.

3. Result and discussion

3.1. Characterization of immobilized lipase

For the preparation of the immobilized lipase, ZIF-8 was first synthesized, and then *Pseudomonas cepacia* lipase was immobilized into ZIF-8 by physical adsorption (Fig. 3).

The structures of synthesized ZIF-8 and PCL@ZIF-8 were characterized by PXRD. The PXRD patterns reveal that the three typical characteristic peaks at 7.36°, 12.7°, and 18° were matched well with the theoretical calculational values (Fig. 4a). There is no significant difference concerning the crystal structure and crystallinity between the ZIF-8 and PCL@ZIF-8 samples, confirming that the ZIF-8 material was successfully synthesized and the PCL@ZIF-8 host could still maintain its framework integrity. SEM were also used to explore morphology. The SEM images show that the morphology of synthesized PCL@ZIF-8 composite is the same as pure ZIF-8 (Fig. 4c and d), indicating that the immobilization process of lipase would not affect the surface morphology of ZIF-8. In order to ascertain that PCL is indeed adsorbed by ZIF-8, the ZIF-8 and PCL@ZIF-8 samples were examined by FTIR. The FTIR results of ZIF-8 ranging from 4000−400 cm^{-1} are shown in Fig. 4b. The absorption bands at 600−900 cm^{-1} may be due to the stretch of aromatic C—H, and 3000–3200 cm^{-1} can be attributed to the stretching of aliphatic C—H by methylimidazole linker [34]. The absorption bands 900−1250 cm^{-1} correspond to the C—N bond stretching. The bands at 1350−1500 cm^{-1} are caused by the stretching of entire methylimidazole ring. The peak at 1580 cm^{-1} corresponds to C═N stretching vibration. Besides, the FTIR spectra of PCL@ZIF-8 demonstrated

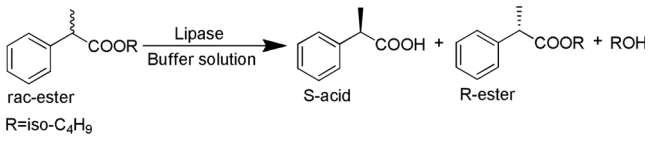

Fig. 1. Enantioselective hydrolysis of (R, S)-2-phenylpropionic acid ((R, S)-2-PPA) ester catalyzed by the enzyme.

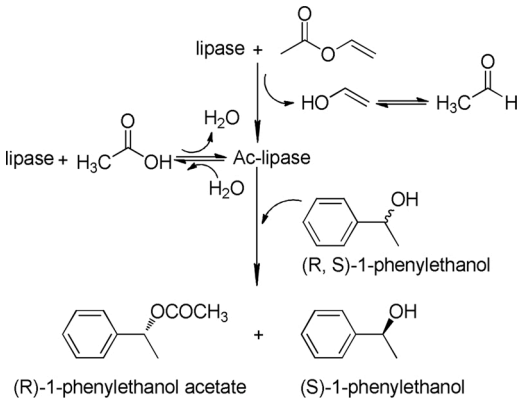

Fig. 2. Enantioselective transesterification of 1-phenylethanol catalyzed by lipase.

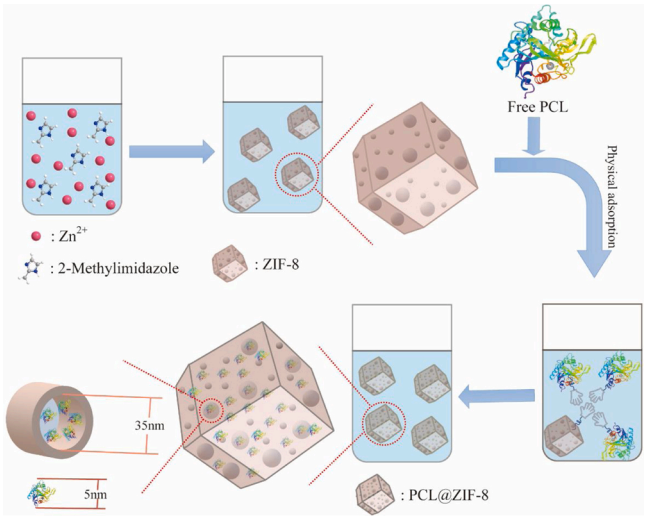

Fig. 3. Synthesis of the Zeolitic imidazolate framework-8 (ZIF-8) and tentative mechanism for the translocation of free *Pseudomonas cepacia* lipase (PCL) into the cavities of ZIF-8.

characteristics peaks of PCL at 1660 cm^{-1}, which belongs to C═O stretching vibration. Therefore, it was confirmed that *Pseudomonas cepacia* lipase was stabilized into ZIF-8 by adsorption. To further verify the immobilization of lipase, N_2 adsorption-desorption isotherms of ZIF-8 and PCL@ZIF-8 were investigated using the surface area analyzer ASAP-2020 at 77 K. The N_2 adsorption-desorption isotherms of ZIF-8 and PCL@ZIF-8 show a characteristic Type IV sorption behavior (Fig. 4e and f) [35]. After adsorption of PCL into ZIF-8, the porosity of ZIF-8 was dramatically decreased due to the residence of enzymes in pores [36]. The BET surface area of ZIF-8 was reduced from 1431 m^2/g to 1253 m^2/g (Table S1). In addition, the t-plot micropore area of ZIF-8 was reduced from 1262 m^2/g to 1100 m^2/g. It was inferred from these observations that a large amount of PCL was adsorbed inside the pores of ZIF-8 (Fig. 3), and an amount of PCL was adsorbed on the surface of ZIF-8 [37]. The pore size distribution of ZIF-8 and PCL@ZIF-8 are shown in Fig. 4e and f, respectively. Compared with ZIF-8, the mesoporous pore number of PCL@ZIF-8 decreased obviously. From Table S1, the total pore volume of ZIF-8 and PCL@ZIF-8 are 1.2991 cm^3/g and 1.2145 cm^3/g, respectively. PCL@ZIF-8 has a smaller total pore volume compared to ZIF-8 due to the embedded PCL molecules, which is consistent with the results reported in the literature [38].

The content of PCL adsorbed into ZIF-8 was determined by thermogravimetric analysis (TGA) method [39]. The TGA curves of ZIF-8 and PCL@ZIF-8 are shown in Fig. 4g. The PCL@ZIF-8 sample shows a deeper drop above 303 ℃ compared to pure ZIF-8, which is attributed to the decomposition of the PCL in PCL@ZIF-8. Compared with the remaining amount of both ZIF-8 and PCL@ZIF-8, the content of PCL in the PCL@ZIF-8 was estimated to be about 16.7 % (200.5 mg PCL/g ZIF-8). From Table S2, compared with other types of supports, such as silica, Silica gel, cellulose, hydroxyapatite and others [40–45], ZIF-8 as immobilization carrier has a higher immobilized capacity due to the high specific surface area and suitable voids.

3.2. Free PCL immobilized into ZIF-8

The effect of immobilization time on immobilized lipase activity and lipase loading amount are shown in Fig. S1. In the initial stage of the reaction, with the extension of the immobilization time, the immobilized lipase activity and lipase loading amount increased rapidly. The relative activity of immobilized lipase and lipase loading amount reached the maximum at 24 h. However, the lipase activity decreased with the enhancement of the immobilization time. It may be due to the weak interaction between free PCL and ZIF-8. Sodium dodecyl sulfate (SDS) is used as a desorbent for the enzyme-MOF complex. After the desorption experiments of PCL@MOF, the catalytic performance of immobilized lipase was rarely maintained only 10 % relative activity, indicating that the lipase is mainly immobilized in the cavity of the MOFs in the form of physical adsorption. Lipase relies on weak van der waals forces and hydrogen bonds to attach to the cavity of the MOFs. A part of free PCL was separated from ZIF-8 after long time soaking. As shown in Fig. S1, it is proposed to control the immobilization time at 24 h.

Since the chemical nature of lipases are proteins, their performances are easily affected by temperature. The effect of temperature on immobilized lipase activity and lipase loading amount were investigated in the range of 5 ℃ to 65 ℃. As shown in Fig. S2, the optimal incubation temperature is 25 ℃. The immobilized amount of lipase did not obviously change with the change of temperature. The immobilized lipase activity increases as the temperature increases within a certain range, and then gradually decreased with further temperature increases. The reason for this phenomenon is that temperature has a dual effect on lipases. Increasing temperature can increase the lipase loading amount, but it will also deform the enzyme and lose its activity after a certain temperature.

3.3. Catalytic performance of immobilized lipase

3.3.1. Hydrolytic activities of free PCL and PCL@ZIF-8

Catalytic experiments were carried out for free PCL and PCL@ZIF-8 to evaluate the activities and enantioselectivities. The schematic diagram of enantioselective catalyzed hydrolysis of 2-PPA ester was displayed in Fig. 5 by using free PCL and PCL@ZIF-8 as catalyst. The activities and enantioselectivities for free PCL and PCL@ZIF-8 were estimated by the conversion rate of (S)-2-PPA ester and enantiomeric excess of (S)-2-PPA for enantioselective hydrolysis reaction. The curves of conversion rate and enantiomeric excess at different times were shown in Fig. 6. It was observed from Fig. 6 and Table 1 that free PCL and PCL@ZIF-8 indicated the same enantiomeric excess of product at the tested times, showing that the immobilization PCL into ZIF-8 maintains the initial enantioselectivity of the free enzyme. We also found that PCL@ZIF-8 demonstrated a faster initial rate of 3.96×10^{-4} mM/s than that of 1.32×10^{-4} mM/s for free PCL as derived from the slope in the first 2 h. In the enantioselective hydrolysis reaction, the catalytic efficiency of the immobilized enzyme is three times that of the free enzyme. After 28 h (Fig. 6 and Table 1), the conversion rate of (S)-2-PPA ester is up to 92 % for PCL@ZIF-8, while it is only 54 % for free PCL. In the same amount of PCL, the immobilization of lipase can significantly enhance the activity of enzyme and improve the initial rate of enantioselective hydrolysis reaction.

By immobilizing PCL into ZIF-8, high selectivity for PCL was not only maintained, but also activity was greatly enhanced. After

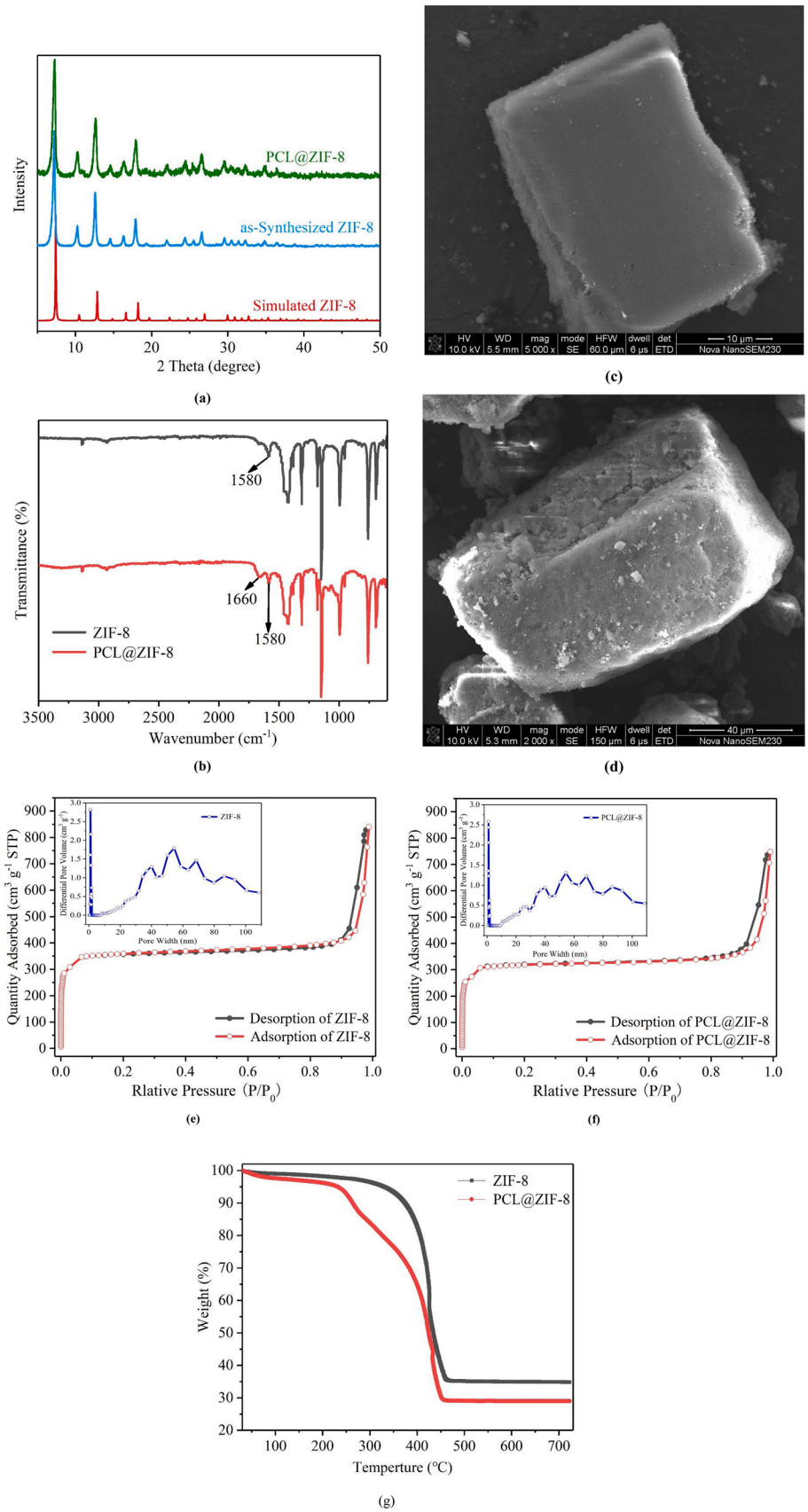

Fig. 4. Characterization: (a) PXRD patterns of simulated Zeolitic imidazolate framework-8 (ZIF-8), as-synthesized ZIF-8, and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8); (b) FTIR spectra of ZIF-8 and PCL@ZIF-8; SEM images of (c) ZIF-8 and (d) PCL@ZIF-8; Nitrogen adsorption-desorption isotherms and Pore-size distribution of (e) ZIF-8 and (f) PCL@ZIF-8; (g) TGA curves of ZIF-8 and PCL@ZIF-8.

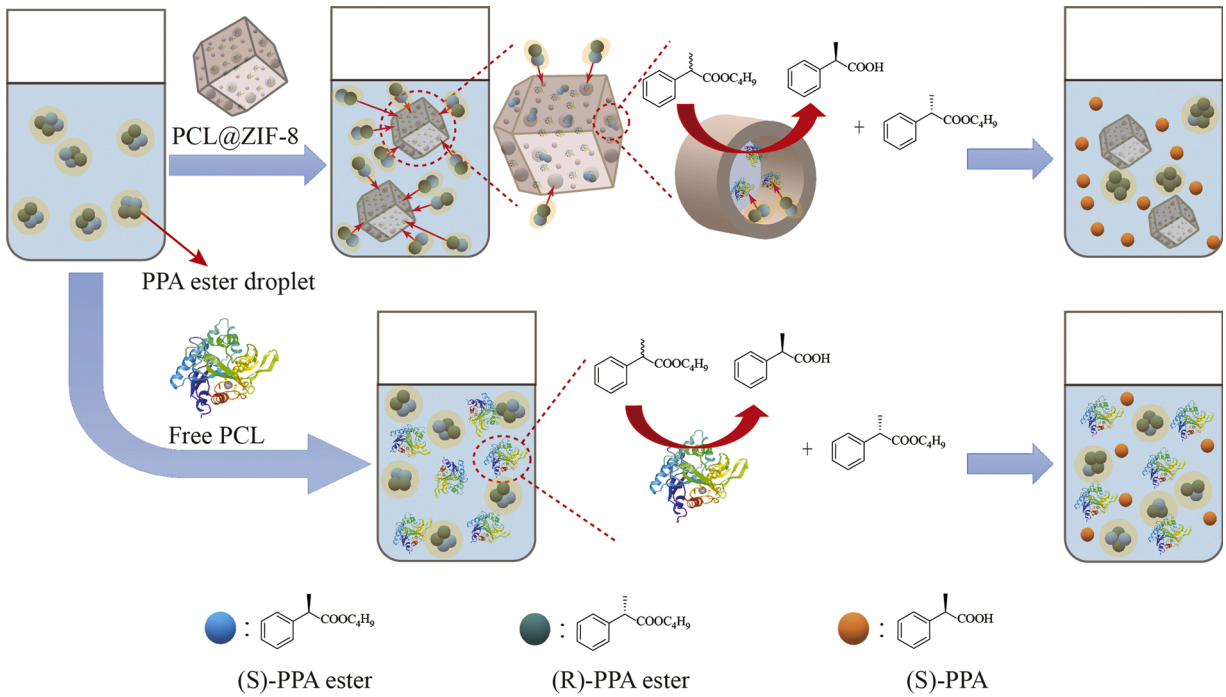

Fig. 5. Schematic diagram of enantioselective hydrolysis of 2-phenylpropionic acid (2-PPA) ester catalyzed by free *Pseudomonas cepacia* lipase (PCL) and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8).

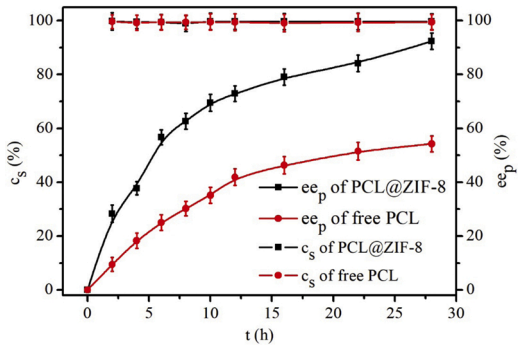

Fig. 6. Hydrolysis activity and selectivity of the free *Pseudomonas cepacia* lipase (PCL) and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8). Conditions: 10 mmol/L (R, S)-2-phenylpropionic acid ester, 2.5 mg/mL free PCL or 12.5 mg/mL PCL@ZIF-8, pH = 6.0, T =328 K.

Table 1
Summary of hydrolytic activity and selectivity of free *Pseudomonas cepacia* lipase (PCL) and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8).

	PCL	PCL@ ZIF-8
Rate (mM/s)[a]	1.32×10^{-4}	3.9×10^{-4}
ee_p (%)[b]	99.46	99.62
c_s (%)[c]	54.225	92.327

[a] Rate of (S)-2-phenylpropionic acid ((S)-2-PPA) ester calculated from the first 2 h.
[b] Final eep after 24 h.
[c] Final c_s after 24 h. Conditions: 10 mmol/L (R, S)-2-PPA ester, 2.5 mg/mL free PCL or 12.5 mg/mL PCL@ZIF-8, pH = 6.0, T =328 K.

immobilization of PCL into ZIF-8, the BET surface area and the t-plot micropore area of PCL@ZIF-8 were still up to 1253 m^2/g and 1100 m^2/g, respectively. 2-PPA ester is difficult to dissolve in water, while the high BET surface area of PCL@ZIF-8 can easily adsorb 2-PPA ester into the cavity of the enzyme-MOF composite material, making the substrate more easily contact with the enzyme to form a complex. Free PCL is a hydrolytic enzyme that is easily soluble in water. Enantioselective hydrolysis of 2-PPA ester enantiomers by free PCL catalyzed was carried out by liquid-liquid interfacial reaction. Therefore, PCL@ZIF-8 has stronger activity than free PCL. Fig. S3 shows that the enantioselective hydrolysis reaction catalyzed by PCL @ ZIF-8 with an optical purity of 99.62 %.

3.3.2. *Transesterification activities of free PCL and PCL@ZIF-8*

Fig. 7 shows the reaction process of enantioselective transesterification of 1-phenylethanol enantiomers by free PCL and PCL@ZIF-8 catalyzed. The activities for free PCL and PCL@ZIF-8 were estimated by the conversion rate of (R, S)-1-phenylethanol for enantioselective transesterification reaction (Fig. 8). It was observed from Fig. 8 that there is no significant difference in the transesterification activity of free PCL and PCL@ZIF-8. At the beginning, free PCL has higher transesterification activity than PCL@ZIF-8, However, the transesterification activity of free PCL decreased significantly after 4 h. This phenomenon is consistent with literature reports [46]. Free PCL is not soluble in the organic phase but is suspended in the organic phase, which is prone to agglomeration in the reaction process. However, PCL@ZIF-8 can effectively prevent the agglomeration of PCL due to the protection of the ZIF-8 with excellent dispersion properties, and still maintain a high transesterification activity. Fig. S4 indicates that the optical purity of the reaction product is up to 99.47 % for enantioselective transesterification reaction by PCL@ZIF-8 catalyzed.

3.3.3. *Reusability of PCL@ZIF-8*

As well known, free PCL is easily soluble in water and agglomerates in organic solvents, making it difficult to reuse. Fortunately, one of the most prominent advantages of the immobilization technology is to improve the reusability of lipase. To investigate the recyclability of PCL@ZIF-8, the enzyme activity and enantioselectivity were determined at different cycles.

Fig. 9 exhibits the recyclability of PCL@ZIF-8 in enantioselective hydrolysis reaction. As the cycle number of PCL@ZIF-8 increases, the conversion rate of (S)-2-PPA ester decreases gradually, but enantiomeric

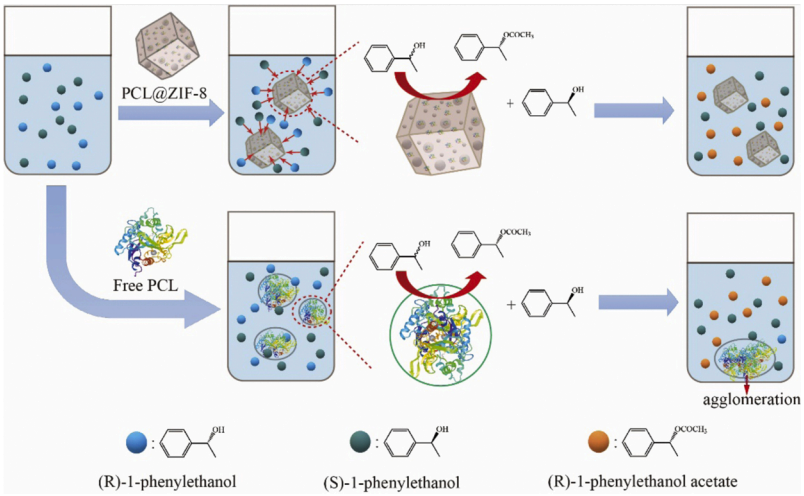

Fig. 7. Schematic diagram of enantioselective transesterification of 1-phenylethanol catalyzed by free *Pseudomonas cepacia* lipase (PCL) and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8).

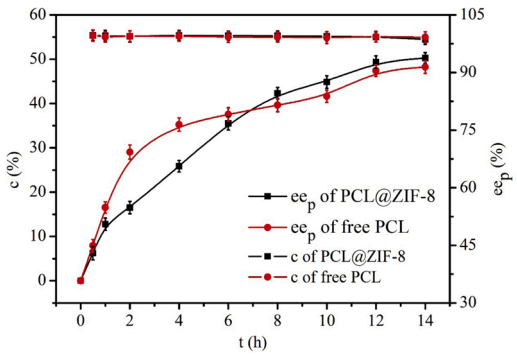

Fig. 8. Transesterification activity of the *Pseudomonas cepacia* lipase (PCL) and free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8). Conditions: 20 mmol/L (R, S)-1-phenylethanol, 100 mmol/L vinyl acetate, 5 mg free PCL or 25 mg PCL@ZIF-8, 3 mL hexane, T =310 K.

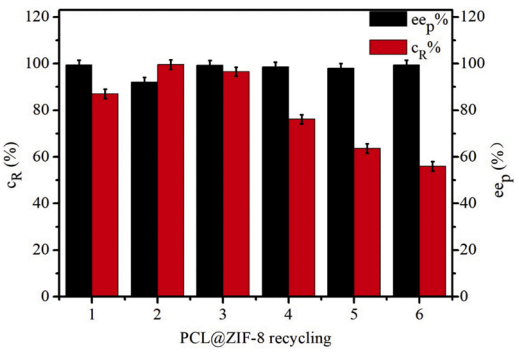

Fig. 10. Transesterification activity and selectivity of free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8) during the recycling use. Conditions: 20 mmol/L (R, S)-1-phenylethanol, 100 mmol/L vinyl acetate, 25 mg PCL@ZIF-8, 3 mL hexane, T =310 K, 10 h.

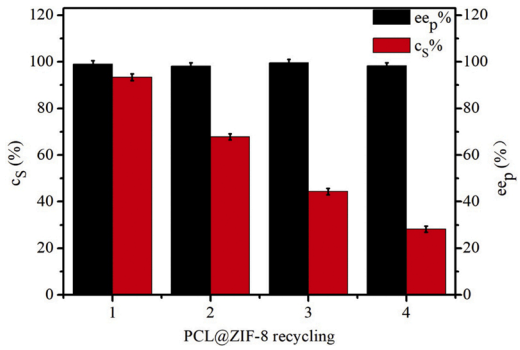

Fig. 9. Hydrolysis activity and selectivity of free *Pseudomonas cepacia* lipase immobilized into ZIF-8 (PCL@ZIF-8) during the recycling use. Conditions: 10 mmol/L substrate, 12.5 mg/mL PCL@ZIF-8, pH = 6.0, T =328 K, t =28 h.

excess of (S)-2-PPA remains almost constant. PCL@ZIF-8 still remained 30.57 % of the initial hydrolysis activity at the fourth cycle. The decay of activity for PCL@ZIF-8 originated from leaching of the PCL immobilized into ZIF-8 during multiple cycles. At the same time, we also investigated the transesterification activity of PCL@ZIF-8 at different cycles. As shown in Fig. 10, the conversion rate of (R)-1-phenylethanol first increases and then decreases. The conversion rate of (R)-1-phenylethanol increased at the second cycle, and we speculated that the reason for the increase of conversion rate was the formation of imprinted enzymes [47]. From the third cycle to the sixth cycle, it was observed that the residual activity gradually declined from 96.48% to 56.01%. Decrease in reusability occurred due to the following reasons: (1) Immersion time in water for too long caused the structure to partially collapse and lipase was separated from ZIF-8. (2) Electrostatic interaction between enzyme and support was destroyed to result in desorption of the immobilized enzyme, including hydrophobic interaction, intermolecular force, hydrogen bonding force, and others. (3) The denaturation of lipase during the reaction process and the diffusion limitation by substrate caused the decay of immobilized enzyme activity [48,49].

In the past decade, some literatures have also reported on the reusability of immobilized PCL in various catalytic reactions [50–55]. However, different characteristics of carrier materials and different types of catalytic reactions have a great influence on the reusability of the immobilized enzyme. It was observed from Table S3 that immobilized PCL can be reused 4–8 times in the catalytic reaction. Compared with other immobilized PCL, the PCL@ZIF-8 also exhibits excellent reusability, maintaining the six cycles. In two reaction system, the recovery rate of the PCL@ZIF-8 was more than 90 % during the recycling use. The advantage of ZIF-8 as a solid carrier for the preparation of immobilized enzymes is that it exhibits high catalytic activity and reusability while maintaining the initial enantioselectivity of the free enzyme. On the other hand, the high specific surface area of ZIF-8 can enhance the adsorption of insoluble substrates, resulting in an increase of reaction rate.

4. Conclusions

In this work, the free PCL was successfully immobilized into the water-stable material ZIF-8 by physical adsorption. Characterization results confirmed that the majority of PCL was adsorbed inside the pores of ZIF-8, and an amount of PCL was adsorbed on the surface of ZIF-8. The PCL@ZIF-8 as effective bio-catalyst was applied for enantioselective enzymatic hydrolysis of (R, S)-2-PPA ester and transesterification of 1-phenylethanol enantiomers. In the catalytic experiments, PCL@ZIF-8 exhibited more excellent enzymatic catalytic performance compared with free PCL. The hydrolysis activity of the immobilized enzyme was three times more than free PCL, and the hydrolysis selectivity was maintained above 99 %. In addition, the PCL@ZIF-8 showed good reusability in both hydrolysis (30.57 % of initial activity, 4 cycle) and transesterification reaction systems (64.38 % of initial activity, 6 cycle). MOFs, which possess simple synthesis, variety and stability, is a kind of potential immobilized carrier for the immobilization of enzymes in the industrial application.

CRediT authorship contribution statement

Jian Ou: Methodology, Investigation, Data curation, Writing - original draft. **Xin Yuan:** Investigation, Writing - review & editing. **Yu Liu:** Writing - review & editing. **Panliang Zhang:** Investigation. **Weifeng Xu:** Investigation. **Kewen Tang:** Conceptualization, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2020.12.017.

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